The Effect of Sex, Age and Ethnicity on Craniofacial Bone Mineral Density

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Abstract

Bone mineral density (BMD) is a strong indicator of bone strength, which is used to discern between individuals with healthy bones and those with metabolic bone diseases such as osteoporosis. The use of DXA is largely centred around measuring key skeletal sites such as the femoral neck and lumbar spine, however some research has used it to measure BMD in the mandible in older edentulous or osteoporotic sample groups. Within craniofacial research, there is little understanding about the mechanisms used to maintain bone mineral density in the craniofacial skeleton as it only experiences small loads. These loads may be generated through mastication or movement of the head and neck. There is a body of research that uses DXA to measure the BMD of facial bones; however, the studies focus on older, edentulous or osteoporotic individuals and do not include comparisons between sexs, age groups or ethnicities in healthy, dentate sample groups. There is a large body of research that has investigated the differences in bite force and muscle activity between sample groups, however little research has been conducted into ethnic differences in these areas. Furthermore, there is very little research that has explored the force-muscle-bone relationship in the craniofacial skeleton. This study aimed to explore that relationship, and investigate how it is affected by differences in sex, age and ethnicity. In particular, the study measured bilateral bite force, jaw elevator muscle activity and mandibular BMD at the ramus and mandibular body. The present study developed a novel bite force device using existing technologies, it also used a new technique of measuring bite force and muscle activity through computer software, which facilitated synchronisation of the data. Furthermore, this study used a new approach to normalising muscle activity data to a submaximal bite force level, a technique used in other disciplines that measure muscle activity but is not often used in

bite force studies. The study also developed a different approach to analysing mandibular BMD from DXA scans, building on the work of previous research. Finally, the study used a new technique for measuring facial dimensions from later photographs rather than radiographs. The present findings indicate no significant differences between males and females in a cohort of young adult Caucasians. The findings indicate a significant effect of age in a cohort of females aged <25yrs compared to >50yrs, but similar differences were not found in males. Significant differences between Caucasian males and African Caribbean males were identified, with particular reference to BMD. Finally, the study reported the significant effect of facial dimensions on the outcome variables bite force, jaw elevator muscle activity and mandibular BMD. The findings are largely concurrent with existing research but also provide new evidence for under investigated areas of research. Overall, this study highlights the use of new techniques for measuring bite force and muscle activity and for analysing BMD from DXA measurements. It has also identified relationships in bite force, muscle activity and mandibular BMD between or within sample groups that have not been reported in previous literature.

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Declaration

I declare that whilst registered as a candidate for the University's research degree, I have undertaken a programme of supporting studies in accordance with the Institutional Practice and Research Degree Regulations. I declare that no material contained in this thesis has been used in any other submission for another award. I declare that I have maintained professional integrity during all aspects of my research degree and have complied with the Institutional Code of Practice and Research Degree Regulations.

Susanna Ruth Crawford

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List of Abbreviations

ACB: African Caribbean British

A-M: Angle to Menton (measurement)

BMD: Bone Mineral Density

vBMD: Volumetric Bone Mineral Density

CB: Caucasian British

C-A: Condyle to Angle (measurement)

C-A-M: Condyle-Angle-Menton (angular measurement)

C-C: Condyle to Condyle (measurement)

CI: Confidence Interval

DXA: Dual energy X-ray Absorptiometry

EMG: Electromyography

sEMG: Surface Electromyography

FRS: Force Sensing Resistor

ICC: Intraclass Correlation Coefficient

IZ: Innervation Zone

LC: Load Cell

LFH: Lower Facial Height (measurement)

MU: Motor Unit

MVC: Maximal Voluntary Contraction

RMS: Root Mean Square

UFH: Upper Facial Height (measurement)

<25yrs: Under 25 years (referring to 18-25 years sample group)

>50yrs: Over 50 years (referring to 50+ years sample groups)

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List of Communications

The communications listed below were derived from the body of research that forms the present thesis.

Chapter 2 Section 2.4 (appendix A):

Paper submitted to the Journal of Oral Rehabilitation,

"Can Masticatory Muscle Activity be Normalised to a Sub-maximal bite Force?"

Accepted November 2014.

Chapter 4 (appendix B):

Abstract and poster accepted to the Bone Research Society Annual Meeting 25th-26th June 2014, Sheffield.

"The relationship between sex and age on mandibular bone mineral density and key anatomical sites as a potential key indicator for the use of facial sports protection".

Chapter 5 (appendix C):

Abstract and oral presentation accepted to the 5th annual International Sports Science and Sports Medicine Conference, 19th-21st August 2014, Newcastle-upon-tyne.

"Facial Bone Mineral Density: A potential indicator for at risk injury groups".

Abstracts to be published in the online edition of the British Journal of Sports Medicine.

Chapter 1: Literature Review

1.1 Introduction to Skeletal Health.

The maintenance of good musculoskeletal health throughout life contributes to the overall quality of life, thus reducing the risk of chronic disease such as obesity and diabetes (Wolfe, 2006), as well as a reduced risk of musculoskeletal injury due to falling and an overall increased life expectancy (Gouveia et al., 2012). There are three fundamental aspects to the health of the musculoskeletal system; hormones, exercise and nutrition (Wolfe, 2006). Hormones play a vital role in the human body for many difference reasons, in terms of skeletal health, increased levels of hormones are most prominent during adolescence and decreased levels of hormones occur during old age, having both a positive and negative consequential effect on the body (Seeman, 2004). During childhood, bones and muscles develop mainly with the assistance of growth hormones, but during puberty they require the combination of thyroid hormone, growth hormone, insulin-like growth factors and sex steroid production, to achieve normal skeletal growth (Bachrach, 2001). Once maturation is achieved, the influence of hormones on the development of the skeletal system remains balanced throughout mid-life, with the exception of pregnancy (More et al., 2001) or disease, as 'external' influences on the endocrine system (Frost, 2001). In later life, the reduction of growth hormones and sex hormones oestrogen and testosterone, particularly in postmenopausal women,

cause a steady loss of muscle quality, muscle mass and bone mineral density (BMD) (Seeman, 2004,; Goodpaster et al., 2006; Seeman, 2008b).

Thus, the importance of an active lifestyle and exercise participation extends beyond cardiorespiratory fitness; it strongly influences the natural balance of the body and aids growth and development during childhood and adolescence (Bielemann et al., 2013; Ortega et al., 2008; Telama et al., 2005). In particular, the skeletal muscles provide a platform for protein metabolism, which aids the functionality of the whole body including the internal organs (Carraro et al., 1990). Exercise promotes protein synthesis in the muscles and regulates insulin sensitivity in the body, which contributes to preventing against chronic diseases and pathological conditions (Wolfe, 2006). Furthermore, the skeletal system itself is positively influenced by exercise; mechanical loading has been found to increase bone mineral density, muscle mass and muscle quality in childhood through to adulthood (Ortega et al., 2008; Sinaki et al., 1998).

In general, nutritional needs remain similar from adolescence to old age. Calcium and vitamin D play the key roles in building and maintaining bone mass and building strong dentition (Tang et al., 2007). As little as 800mg (Ettinger, 1992) of dietary calcium a day (the equivalent of 3 servings of calcium rich foods such as milk, yoghurt or leafy green vegetables), is enough to improve bone mass in early adulthood and maintain it throughout maturation. Increased levels of calcium intake (1,000-1,200mg per day) are necessary in the elderly due to poor intestinal absorption, but even increased levels will only help to moderately slow the rate of bone loss. Vitamin D aids and regulates the absorption of calcium and phosphorous,

another key mineral in bone and dental health (Holick, 2002). Vitamin D deficiency is caused by poor diet, lack of exposure to sunlight and poor intestinal absorption (common in the elderly or infirm). It manifests as aches, pains and weakness in the muscles and bones, and can aggravate conditions such as Osteoporosis, Osteomalacia or Rickets in children. Thus, the relationship between the muscle and bone interaction are key throughout life. The following section will focus on both bone and muscle independently, as well as a concomitant pair in relation to physical activity/loading.

1.2 Bone

1.2.1 The Microarchitecture of Bone

Human bone consists of two principle components; an organic material (Collagen, specifically Type 1) and a mineral constituent Hydroxyapatite ($Ca_5(PO_4)_3(OH)$), which is a form of calcium phosphate (Currey, 2002). Bone tissue also contains water, nutrients and a blood supply, which allows it to grow and adapt to external influences (Currey, 2002). In terms of weight, the human bone matrix comprises of approximately 65% Hydroxyapatite and 35% organic compound (Pearson and Lieberman, 2004), of which 85-90% is Collagen Type I and the remainder are non-collagenous proteins (Currey, 2002). The organic collagen provides elasticity and toughness to the bone structure, which allows for flexibility and deformation under loading, without fracture (Seeman, 2008a). The mineral content, attached to the organic structure, is very dense in comparison and provides structural stiffness (Rho et al., 1998). The varied levels of mineral content in bone are thought to be the key to differences in mechanical stiffness between specimens, thus showing that the material parameters in bone influence its mechanical performance (Currey, 2003). Therefore, the combination of collagen and mineral produce a strong, structured tissue that is both tough and stiff and can absorb forces along multiple planes (Seeman, 2008a). At a material level bone is heterogeneous, which means its structure or properties are non-uniform in nature. In addition, bone is anisotropic

(Rho et al., 1998), which describes its properties as directionally dependent, rather than expressing identical material properties in both its longitudinal and transverse plane (Buckwalter et al., 1995). The heterogeneity of bone allows for growth, development and adaptation to occur throughout life and in response to injury or disease (Frost, 2001).

Bone Cells

At the nanostructure there are three notable cells that operate within bone tissue, osteoblasts, osteocytes and osteoclasts. Their function is to work in harmony to resorb, deposit and remodel bone, through mechanical strain stimulation. Osteoblasts are bone-forming cells that synthesise new collagen matrix, then deposit mineral crystals within and between the fibres (Rho et al., 1998). Osteoblasts originate in a number of key areas within the bone, including the periosteum (outer surface) and endosteum (inner surface) (Pearson and Lieberman, 2004), where a large portion of bone deposition occurs (Weiner et al., 1999). Osteocytes are the primary cells of mature bone, they are located in small spaces called Lacunae and help to regulate the metabolic and sensory functions of bone (Currey, 2003). Osteocytes are of osteoblast lineage; they have been depicted as osteoblasts that have become trapped in the newly deposited bone (Ferretti et al., 2002; Pearson and Lieberman, 2004), or rather are slow working osteoblasts which are passively buried by other osteoblasts actively producing more bone matrix (Franz-Odendaalo et al., 2006). Finally, Osteoclasts are large multinucleated cells, that are responsible for

resorbing damaged or old bone, as such they are aggressive in nature. The actions of osteoclasts and osteoblasts are usually referred to as resorption and deposition, respectively. Both resorption and deposition phases can occur independently within bone tissue, as a response to loading/non-loading (Frost, 2003), but they also occur in sequence during 'remodelling'. Osteoblasts and osteoclasts form a Basic Multicellular Unit (BMU) which resorbs old bone and deposits new bone sequentially (Frost, 2003) (Figure 1.1).

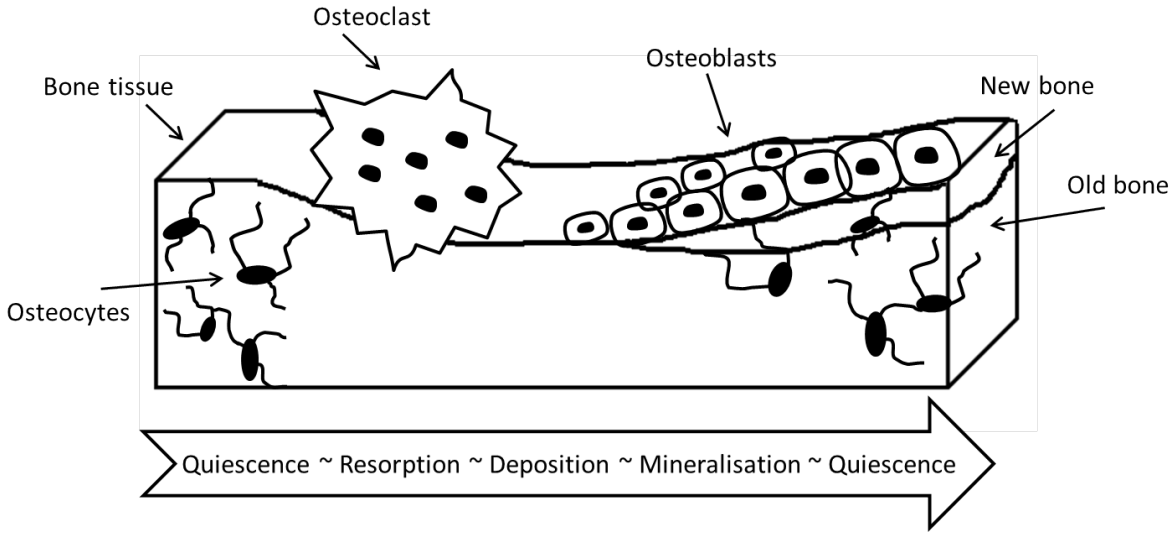


Figure 1.1: Schematic diagram of the remodelling process of bone tissue.

Bone Structure

Bone can be deposited in two different forms named Woven and Haversian (also named Lamella). Woven bone forms the embryo *in utero* and is slowly replaced by mature Haversian bone after birth as the skeleton grows (Buckwalter et al., 1995). Woven bone is also deposited very quickly in response to injury, rapid bone

deposition at the growth plates or disease (Gorski, 1998), and although it is still compact, it is very irregular in structure. For the most part, the post pubertal skeleton is comprised of slowly deposited, structured Haversian bone (Buckwalter et al., 1995). The human skeleton comprises of two types of Haversian bone tissue that combine to form whole bones: i) cortical bone, also referred to as compact bone ii) and cancellous bone, also referred to as trabecular or spongy bone (Currey, 2002). Cortical and cancellous bone tissue have the same mineral and organic components and are replenished by osteoblast and osteoclast activity, through the same modelling and remodelling processes. However, cortical and cancellous bone differ considerably in their structure (Currey, 2002; Seeman, 2008a). Cortical bone is a dense structure that most commonly forms the outer shell of whole bones, with a higher percentage found within the diaphysis region. This provides support and strength, particularly to weight bearing bones such as the femur, which supports a large amount of muscle and bone mass (Seeman, 2008a), as well as maintaining skeletal shape (Currey, 2003). It consists of osteons, which are a multi-layered cylindrical arrangement of mineralised collagen fibrils. These layers of fibrils are called lamellae, they are positioned concentrically around minute canals that house blood vessels and nerves, as a form of protection (Wagermaier et al., 2006). Lamellae are also found around and between the osteons, known as circumferential and interstitial lamellae (Buckwalter et al., 1995). This provides further density and improved structure to the Haversian system (Figure 1.2). In long bones, osteons run parallel with the long axis, therefore the dense strips of collagen in the fibrils (of circumferential and interstitial, as well as concentric lamellae) are structurally placed

to absorb compression in weight bearing bones (i.e. the femur) (Rho et al., 1998). Recent research suggests that although lamella are essentially constructed this way, the layers of fibrils are multidirectional (Weiner et al., 1999), with interwoven fibres (Rho et al., 1998), which provide additional strength and structure in an anisotropic fashion (Wagermaier et al., 2006).

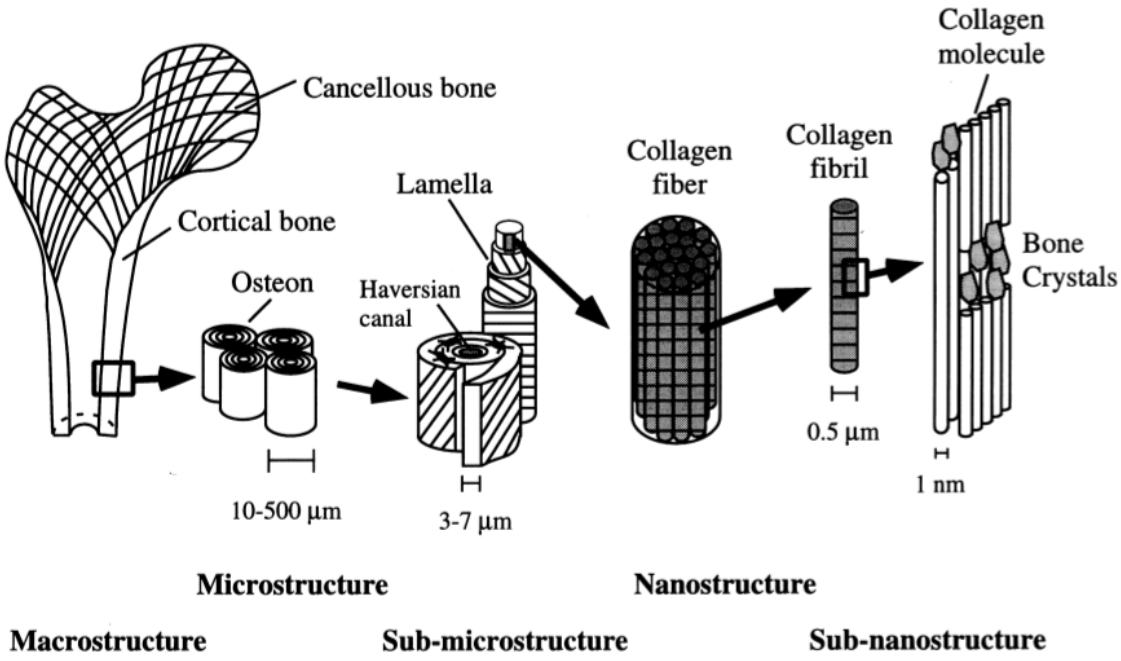


Figure 1.2: Structural composition of bone tissue.

(Original figure obtained from Rho et al. (1998))

In comparison, cancellous bone is lighter and less dense, its structure is an interconnecting framework of bony struts named trabeculae. The fine rods of the trabeculae are formed of lamella fibrils in the same fashion as compact bone (Ferretti et al., 2002). However, cancellous bone is less uniform and has greater

porosity, and is thus associated with changes in bone density, particularly osteoporosis. The cancellous trabeculae are found connected to the inside of the compact bone cortex, with a higher abundance specifically within the epiphyseal sections of long bones and within the main body of the vertebrae (Frost, 2001). The cortical and cancellous tissue and the bone marrow they surround are synchronous, they facilitate and share the osteogenic cells and the blood supply (Buckwalter et al., 1995). As in all Haversian bone tissue, the osteogenic cells work on the surface of the trabeculae to improve their size and thickness (Pearson and Lieberman, 2004). This process most commonly occurs in response to mechanical strains/stimuli on the bone by muscle interaction (Frost, 2003) and reinforces the strength of the whole bone structure, whilst the spaces between the trabeculae reduce the overall weight of the bone (Currey, 2002).

Whole Bone Structure

The mechanical reliability of whole bones not only incorporates the material properties of the tissue, but also the architecture of the whole bone (Currey, 2003). Based on shape alone, bones can be categorised as such: long bones, short bones and flat bones (Buckwalter et al., 1995). Long bones include limb bones such as the femur, tibia and humerus, but also small bones such as the metacarpals and metatarsals. Short bones include carpals, tarsals and vertebrae, whilst flat bones comprise of the scapula, cranial vault and the mandible. Each bone in the skeleton is specifically situated, and thereby its construction is well adapted to the mechanical

demands placed upon it (Currey, 2002). Long bones are longer than they are wide, they have growth plates at either end (which close at maturation) named epiphyses and they comprise of cancellous bone encapsulated in a tubular structure of cortical bone (Pearson and Lieberman, 2004). Short bones are roughly as long as they are wide and hold a variety of cuboid-like shapes. Short bones comprise of a greater amount of cancellous tissue which is contained within a much thinner cortex than that of long bones, this makes them less dense and lighter in comparison (Buckwalter et al., 1995). The vertebral bones in particular are oval in shape, tapered in size towards the neck and their high cancellous bone content allows them to act as springs or shock-absorbers in the axial skeleton (Seeman, 2008a). Flat bones tend to be non-tubular in shape, with one dimension that is much greater than the other two (Buckwalter et al., 1995). In general flat bones function as protection for vital organs and attachment sites for muscles (Currey, 2002). Flat bones comprise of a thin layer of cancellous bone sandwiched between two layers of dense cortical bone (Rho et al., 1998), in the cranium this is often referred to as the diploe. The following section will refer to bones in general, and Section 1.2.2 'The mechanical response of bone' will consider weight-bearing bones. Facial bones, particularly the mandible, will be discussed in Section 1.5 'The Craniofacial Skeleton'.

Bone, Genetics and the Endocrine system

Osteoblast activity is regulated by the endocrine system, particularly parathyroid hormone (PTH), 1,25 hydroxyvitamin D, calcitonin and sex hormones (Pearson and

Lieberman, 2004). Children are capable of rapid increases in bone mass and size, particularly of the appendicular skeleton, up to puberty (Seeman, 2004; Bachrach, 2001). During puberty, when the body is secreting larger amounts of sex hormone, changes in growth patterns occur (Seeman, 2004); together with sex hormones (particularly oestrogen and testosterone), the body regulates thyroid hormone, growth hormone, and insulin-like growth factors (Bachrach, 2001). Concurrently, the body draws on parathyroid hormone and 1,25 hydroxyvitamin D, which are essentially mineral-building or mineral-preserving hormones that stimulate osteoblast activity (Pearson and Lieberman, 2004). The regulatory role of oestrogen further explains the differences between males and females during and after puberty; oestrogen in particular plays an important role in skeletal changes, as it regulates osteoblast (and indirectly osteoclast) activity in both males and females. However, the level of oestrogen in females inhibits increased bone size (Seeman, 2004), which specifically results in stimulated endosteal apposition but impeded periosteal apposition (Eastell, 2005), this leads to bones which have smaller circumferences than males, but an improved cortex thickness (Seeman, 2008a). Conversely, the male sex hormone androgen produces increased bone size at the periosteal surface (Seeman, 2004), therefore as males enter puberty later and experience a longer spell of accelerated growth than females (Eastell, 2005), their appendicular bones grow larger in circumference and thicker than that of females (Seeman, 2004; Seeman, 2008a; Hind et al., 2012). During adulthood the influence of hormones on the skeletal system remains balanced in males, but varies in females due to age of menarche and menstrual cycle length (Cooper and Sandler, 1997),

further changes may be experienced during pregnancy (More et al., 2001). Long term diseases in either males or females can also result in skeletal changes (Frost, 2001). Growth hormones in particular remain constant throughout adulthood and even in later life, but growth hormone secretion can be negatively affected by other hormone levels with the onset of ageing. This results in lower circulating levels of growth hormone and hinders protein synthesis in the muscles (Rudman et al., 1981). The large reduction of sex hormones, particularly in postmenopausal women, causes a rapid and sometimes chronic reduction in bone mineral density, as a result of increased BMU activity at the endosteal surface. This intensifies bone resorption, but postmenopausal women are deficient in osteoblasts required for new bone deposition. Conversely, elderly males experience a slow, steady loss of bone mineral density (Seeman, 2004; Seeman, 2008b; Goodpaster et al., 2006). This is one factor as to why females are more prone to osteoporosis in later life, compared to that of their male counterparts.

In relation to ethnicity, significant ($p=0.0011$) (Wright et al., 1996) differences have been found in the level of circulating oestrogen (specifically 17 β-estradiol) and growth hormone between Caucasian (108±11 pmol/L) and Black (162±12 pmol/L) adult males. This has been significantly linked to BMD at key skeletal sites (femoral neck, lumbar spine, trochanter and total body BMD) (Wright et al., 1995). However, these differences in oestrogen and growth hormone between ethnicities were not replicated in a similar study comparing premenopausal Caucasian and black adult females, despite significant differences in BMD (Wright et al., 1996). Gilsanz et al. (1991) reported vertebral trabecular densities of 202±21mg/cm^3 in a Black cohort

versus 166±19mg/cm^3 in a Caucasian cohort of female adolescents at tanner stage IV by quantitative computer tomography. Their findings equate to a mean difference of 24%. The reasons for ethnic differences during puberty is uncertain but may also be related to circulating levels of hormones, as seen in adult populations (Wright et al., 1995). Black cohorts have been found to have a lower sensitivity to oestrogen, therefore bone growth is inhibited less in both men and women (Seeman, 1998). Ethnicity differences reflect genetic differences as well as environmental influences on the skeletal system. Variations in bone mineral density across many different ethnicities may reflect variations in diet, climate and activity levels, but crucially a variation in genetics (Pollitzer and Anderson, 1989). Ethnic differences are further complicated by sex differences in BMD gain during puberty, and BMD loss due to ageing (Seeman, 1998), which indicates that BMD is significantly higher in males than females throughout life, regardless of ethnicity (Looker et al., 2009). Bone mineral density differences during puberty indicate higher femoral neck BMD values in Black populations (0.9g/cm^2 females) with Caucasian (0.83 g/cm^2 females) and Hispanic (0.83g/cm^2 females) demonstrating similar values and Asian (0.81g/cm^2 females) populations the lowest (Wang et al., 1997). These findings are also reflected in adult BMD differences between ethnicities, which shows Black populations have the highest total mean body BMD (1.19g/cm^2 females, 1.28g/cm^2 males) followed by Caucasian (1.1g/cm^2 females, 1.2g/cm^2 males) and Hispanic populations (1.1g/cm^2 females, 1.19g/cm^2 males) (Looker et al., 2009). Despite clear differences in BMD and fracture rates in the elderly between ethnicities, it is still not fully understood the extent to which genetics influences skeletal parameters (Looker, 2002). As

shown in twin studies, high correlations in BMD between female monozygotic twins, both pre- and post-menopause, indicates a high heritability of bone mass (Slemenda at al., 1991). Overall, these findings suggest that there is a strong genetic influence on bone modelling and remodelling, however the genetic sequence can only promote or hinder bone formation and growth factors; it is the application of mechanical strain that instigates modelling or remodelling. The structure and adaptation of the human skeleton is a collection of complex, multifaceted processes, which are affected by many genetic, hormonal and not least, mechanical factors. The latter has arguably the most significant effect on changes to bone health throughout life (Frost, 2002). Thus, the following section will explore the mechanical response of bone, as the area of interest within the present study is the BMD of the hip, lumbar spine and the mandible, which are areas where loading occurs either as the result of a direct or indirect response.

1.2.2 The Mechanical Response of Bone

The whole structure of bone determines the loads that can be tolerated, but equally the loads applied to the bones determine the changes that occur within the bone tissue and the whole bone structure (Hsieh et al., 2001). Mechanical responses were first reported by Julius Wolff, who explained the distribution and orientation of trabeculae in the proximal femur, in response to mechanical strains (Ruff et al., 2006). Wolff's law, as it became known, originated as a mathematical model and although it stood as the principle theory on bone adaptation for decades, it has long since been built upon with new discoveries, in part, disproving some of Wolff's theory (Pearson and Lieberman, 2004; Ruff et al., 2006). Recent research acknowledges the complexities of bone mechanics, bone homeostasis and the influence of genetics (Frost, 2003).

Mechanisms and Mechanics of bone adaptation

The osteogenic process of sensing and responding to external loads is thought to encompass several stages or mechanisms (Pearson and Lieberman, 2004) but is one of the least understood systems within bone (Robling et al., 2002). Mechanical energy is converted to electrical and /or biochemical signals through a process termed mechanotransduction (Burger and Klein-Nulend, 1999). The transduction of biological forces into cellular responses are key to many areas of the human body

(Turner and Pavalko, 1998). This is important because mechanosensitivity modulates a vast array of physiological processes, which result in either growth and development or, through dysregulation, in disease (Orr et al., 2006). Mechanical adaptation in bone, at a cellular level, requires the biological system to sense mechanical stimuli/loading, then communicate the information to an effector cell, which either deposits new bone or resorbs old bone (Burger and Klein-Nulend, 1999). Turner and Pavalko, (1998) proposed that the mechanotransduction involves four stages or processes. Firstly, mechano-coupling, where the mechanical force signal is received by a local sensor cell in the bone tissue. Secondly, biochemical coupling where the transduction of local mechanical force into biochemical signalling occurs, which results in gene expression or protein activation. Thirdly, the transmission of the signal from the sensor cell to the effector cell. Finally, the response of the effector cell in the bone tissue. Despite the existence of research to support each of these stages, the process of mechanotransduction is still not fully understood. Postmiotic osteocytes (osteoblasts that have been trapped in the bone matrix) are commonly believed to be the primary mechanotransducing cell in the bone tissue (Turner and Pavalko, 1998; Pearson and Lieberman, 2004; Orr et al., 2006). Osteocytes are appropriately placed in the bone to sense loading and communicate several cellular processes, including energy metabolism, gene activation, growth factor production and matrix synthesis, by transporting signals through the lacuno-canalicular network (Burger and Klein-Nulend, 1999). The non-mineralised matrix surrounding the osteocytes in their lacunae is more porous than mineralised bone, which allows permeable canalicular to transfer nutrients, small

molecules and waste throughout the bone matrix (Orr et al., 2006), resulting in a living, adaptable bone structure with complex pockets of porosity (Burger and Klein-Nulend, 1999). Osteocytes are connected to as many as 60 cytoplasmic processes (Cowin, 2002) running through the canalicular in the bone surface and deep in the bone cortex, which connects osteocytes, osteoblasts and preosteoblastic cells (Franz-Odendaal et al., 2006). The compressive and tensile forces acting on the bone tissue during mechanical stimuli cause interstitial shear forces to occur, through a change in the pressure of the fluid flowing in the canalicular (Orr et al., 2006). Research suggests that osteocytes are especially sensitive to fluid shear stresses, they sense the change in interstitial fluid flow, and respond with signals that stimulate osteoblast and osteoclast activity (Cowin, 2002; Orr et al., 2006). Further studies have suggested that the flow of interstitial fluid induces a drag force surrounding the osteocyte process, causing a greater level of strain than the fluid shear forces (Orr et al., 2006). However, Fritton et al. (2000) stated that the exact nature of these processes are far from understood and more investigation is required, particularly with regard to strain magnitude, frequency and resulting deformation. The response to mechanical loading is performed by the effector cells, the osteoblasts and osteoclasts, that travel to the specific site to begin modelling or remodelling (Cowin, 2002). The signals caused by mechanical loading must contain specific, dynamic information so that the magnitude and frequency of the signal exceeds a threshold set by the cell (Frost, 2003). Additionally, for an osteogenic response to occur, the bone cell must be receptive of mechanical signals and able to transmit information (Robling et al., 2002). This may partly explain the decrease in

osteoblast activity due to ageing, when less active receptor cells are present in the tissue to transmit information to the effector cells (Turner and Pavalko, 1998).

Further to the mechanotransduction process, research proposes strain thresholds of use and disuse within bone tissue (Frost, 2003). It is acknowledged that mechanical strains above a certain threshold induce deposition of bone tissue whilst mechanical strains below a certain threshold cause resorption of bone tissue, through increased osteoclast activity (Frost, 2002; Pearson and Lieberman, 2004). This is the basis of the mechanostat theory, proposed by Harold Frost, which encompasses modelling and remodelling mechanisms and thresholds with regard to loading (Schoenau, 2005) (Figure 1.3). Furthermore, the mechanostat theory (and the Utah paradigm into which it evolved) acknowledges and incorporates the systemic effects of genetics and the endocrine system on strain thresholds; alongside the localised effect of loading on bone modelling (Turner and Pavalko, 1998; Frost, 2004; Schoenau, 2005). The frequency and magnitude of the loads exerted on bone define the level of response. In young adult bone, loads causing <400 microstrain ($\mu\varepsilon$) induce remodelling and bone loss, loads causing 1,000-1,500 $\mu\varepsilon$ instigate modelling, loads causing >3,000 $\mu\varepsilon$ cause micro-damage and very high loads of 25,000 $\mu\varepsilon$ result in fracture (Frost, 2003). Micro-damage and fracture response occur in the plastic region, when deformation exceeds the threshold (or postyield) and some permanent changes, or damage occur as a result (Currey, 2002; Winwood et al., 2006). Micro damage may be easily healed and the bone tissue strengthened again at a cellular level (Turner, 1998), provided the subsequent strain levels are below the damage threshold (Frost, 2003).

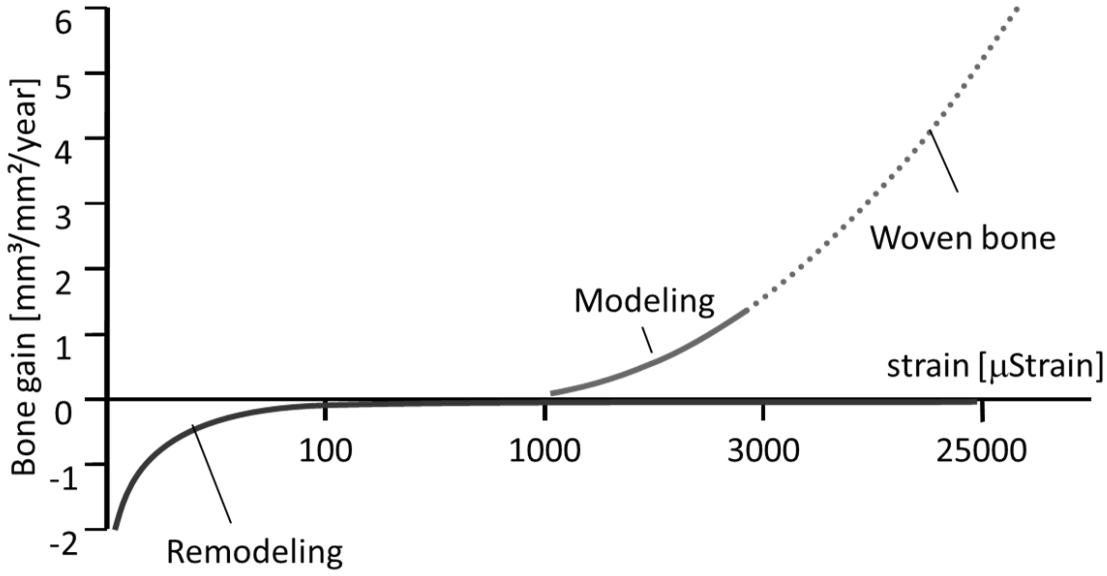


Figure 1.3: Mechanostat theory of bone modelling and remodelling.

(Original figure obtained from Frost (1987)).

The typical peak loads experienced in an active everyday life are less than the modelling threshold but more than the remodelling threshold (Frost, 2003). This is the predicted equilibrium range in which loading has neither a modelling, nor remodelling stimulus on bone (Perason and Lieberman, 2004). An individual's modelling and remodelling thresholds are largely influenced by genetics and embryonic development (Lovejoy et al., 2003) as well as linking to the genetically determined endocrine system which secretes bone influencing parathyroid- and sex hormones (Burger and Klein-Nulend, 1999; Lanyon and Skerry, 2001). Despite a strong systemic influence, mechanical adaptation due to loading remains the key to bone modelling and remodelling, with changes in strain thresholds occurring following intense bouts of loading. Robling et al. (2002) reported a significant

increase in areal BMD and bone mineral content (BMC) in rat ulnas, following repeated bouts of loading over 16 weeks. Specimens subjected to continuous bouts of loading showed less improvement in biomechanical and structural properties, than those that experienced shorter bouts, separated by rest periods. These findings are attributable to the desensitisation of the bone cells to mechanical stimulation (Hsieh et al., 2001) and they support the notion that local bone formation is more efficiently attained through discrete loading, particularly on the periosteal surface (Hsieh et al., 2001; Robling et al., 2002).

An expansion of the mechanostat theory, which encompasses new research on bone mechanical responses, and incorporates clinical and surgical findings, was developed. This new body of research has been referred to as The Utah Paradigm (Frost, 2001). The paradigm expands to encompass all contributable research into the physiological, anatomical, pathological, clinical and homeostasis differences in bone throughout life. Indeed, the Utah Paradigm is already extensive, with 21 listed 'Clinical Phenomena' that can be explained by research. Two such questions the Utah Paradigm can plausibly explain are i) "why strong muscle usually associates with strong bone" and ii) "why chronic muscle weakness associates with osteopenia". Both issues highlight the importance of understanding the muscle-bone relationship, and its pivotal role in protein metabolism for bone homeostasis, as well as applying localised strain for increased bone formation (Wolfe, 2006). However, without regular peak muscle contractions, bones will not experience strains great enough to maintain or form new bone tissue (Schoenau, 2005).

Thus, the following section will review the microarchitecture of muscle and the muscle-bone interaction.

1.3 Muscle

1.3.1 The Microarchitecture of muscle

Muscles are molecular structures that convert chemical energy stored in the body into force (Enoka, 2008), for which their basic structure is suitably designed. Muscle has four main tissue properties: irritability, contractibility, extensibility and elasticity. Irritability simply means that the tissue can be stimulated by an external factor, which induces a characteristic action or function. In muscle tissue, that function is contractibility (Hamill and Knutzen, 2006), or a temporary shortening in length due to temporary changes in the microarchitecture. Muscles demonstrate elasticity by returning to their original length once tensile forces are removed, subsequently exhibiting viscoelastic properties and viscoelastic/plastic deformation when stretched (Weppler and Magnusson, 2010). Viscoelastic deformation is dependent on the magnitude and duration or frequency of the stretch, therefore extended periods of stretch would induce extensibility in the muscle tissue. However, Weppler and Magnusson, (2010) suggest that muscle's extensibility is restricted by its innate elasticity. Therefore, changes in muscle extensibility following short term (<8 weeks) stretching programmes are more likely to be due to changes in a subject's sensation (Magnusson et al., 1996) or tolerance to uncomfortable sensations (Folpp et al., 2006).

Microscopic Muscle and the Sliding Filament Theory

At a microscopic level, muscle tissue comprises of fine strands that run the whole length of the muscle, known as myofibrils (Huxley, 1969). The myofibrils are bundled together to form muscle fibres which are individually covered in a connective tissue called the Endomysium (Borg and Caulfield, 1980). Despite being the smallest components of the muscle tissue, the myofibrils contain the key to muscular contraction (Figure 1.4). They contain contractile proteins that are fundamental for muscle tissue irritability, and therefore instigate whole muscle contraction when stimulated (Huxley, 2004). The contractile protein myosin forms thick, repeated striations, which are situated opposite a thin polypeptide contractile protein actin (Li et al., 2014). The formation of these two proteins creates a chain of bonded units called sarcomeres, which host the genesis of muscular contraction (Huxley, 2000). Muscle tissue requires an external stimulus to instigate contraction, this is provided by motor neurons which innervate muscle tissue through electrical signalling (Goldspink et al., 1974). The central nervous system in the brain sends signals to the motor neurons in the muscles via the spinal cord; each motor neuron attaches to the spinal cord and a number of muscle fibres that it innervates known as a motor unit (MU) (Merletti and Parker, 2004). The signal sent from the neuron to the muscle is called the action potential, if this is large enough to reach the stimulation (or excitation) threshold, then all sarcomeres in that MU contract (Merletti and Parker, 2004). The transformation of an axonal (spinal) action potential into a muscular potential is collectively termed a neuromuscular propagation (Enoka, 2008). The

most prolific theory used to explain the contraction of sarcomeres is called the sliding filament theory (Huxley, 2004; MacIntosh et al., 2006). Essentially, neuromuscular propagation is a form of signal transduction, converting chemical energy into mechanical energy, and the key to this form of cell signalling in the body (not just the muscles) is calcium ions (Ca^{2+}) (Clapham, 2007). The action potential causes a depolarisation of the exterior membrane of the muscle fibres, which in turn causes the release of intracellular Ca^{2+} (Pertersen et al., 2005). The Ca^{2+} then diffuse into the protein filaments and bind to Ca^{2+} regulatory sites (Baylor and Hollinworth, 2011). This signal causes the myosin filament to slide along the actin filament; each myosin molecule has two heads (or crossbridges) which connect to specific binding sites on the actin filament and consequently contracts the sides of the sarcomere by drawing the actin filament in towards the centre of the myosin (Figure 1.4) (Barclay et al., 2010).

The simultaneous sliding of many thousands of sarcomeres together changes the length and force of the whole muscle tissue. Therefore, a greater number of cross bridges would produce a greater force (Huxley, 2000; Huxley, 2004; Li et al., 2014). In normal musculoskeletal fibres stimulated by an action potential, the release and binding of Ca^{2+} is large and rapid (Baylor and Hollingworth, 2011), commonly lasting 0.01s (Petersen et al., 2005) and each Ca^{2+} release is specific to the cell and tissue type (Clapham, 2007). Furthermore, through mechanical modelling, Li et al. (2014) reported that sarcomere shortening speed is dependent on the modulation of myosin and cannot infinitely increase. Motor units exhibit striking differences among

themselves in terms of size, speed of contraction and biochemical capabilities, which suggests that each MU is purposely suited to its task (MacIntosh et al., 2006).

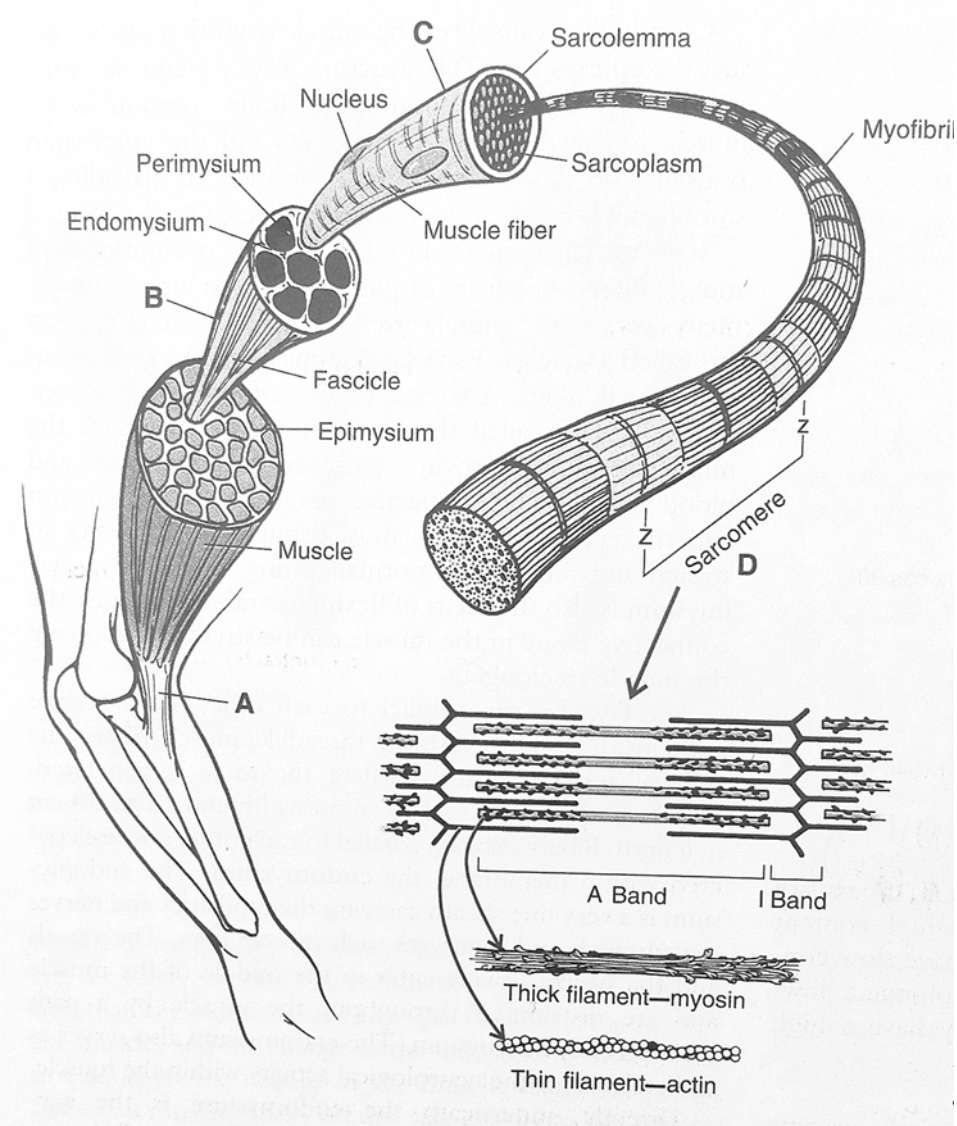


Figure 1.4: Structure of muscle tissue.

(Original figure obtained from Hamill and Knutzen (2006), p70).

The Whole Muscle: Structure and Contraction

Bundles of muscle fibres (of varying numbers up to 200) create a structure known as a fascicle, which is protected by the perimysium, a dense connective tissue largely composed of Type I collagen (Light and Champion, 1984), which allows blood vessels and nerves into the muscle body between the fascicles (Figure 1.4) (Borg and Caulfield, 1980). Each MU innervates a group of muscle fibres, which stretch across several fascicles within the muscle tissue. There can be anything up to 2,000 fibres included in one MU, depending on the size and function of the muscle (MacIntosh et al., 2006). MUs differ in electrical properties and contractile properties (i.e. speed, force generation, fatigue resistance), therefore an increase in force requires an increase in MU recruitment, this is usually sequential, following an orderly pattern (Hamill and Knutzen, 2006). The whole muscle structure comprises many fascicles and is protected by a connective tissue the Epimysium, which is estimated to account for only 1.2% of the total muscle weight (Light and Champion, 1984). This also serves to connect the whole muscle to the skeleton through a tendon or aponeurosis and provides structure to the muscle to enable movement. Together the three connective tissues (Endomysium, Perimysium and Epimysium) influence the mechanical properties of the muscle, including its extensibility and elasticity, which is crucial for facilitating movement thereby having an effect on the underlying bone tissue (Borg and Caulfield, 1980; Folpp et al., 2006).

The Muscle-Bone Interface/Unit

Bones are primary attachment sites for muscles, either directly or via tendons and aponeuroses (Pearson and Lieberman, 2004). The muscular connective tissues, particularly the epimysium and perimysium, join to the tendon or aponeurosis, which in turn attaches directly to the bone (Figure 1.5) (Light and Champion, 1984; Cowin, 2002; Pearson and Lieberman, 2004). This process creates a simple pathway for force transmission and stimulates bone cell activity (Cowin, 2002), having both an effect on the bone density of the tissue and also effecting the anatomical shape during growth.

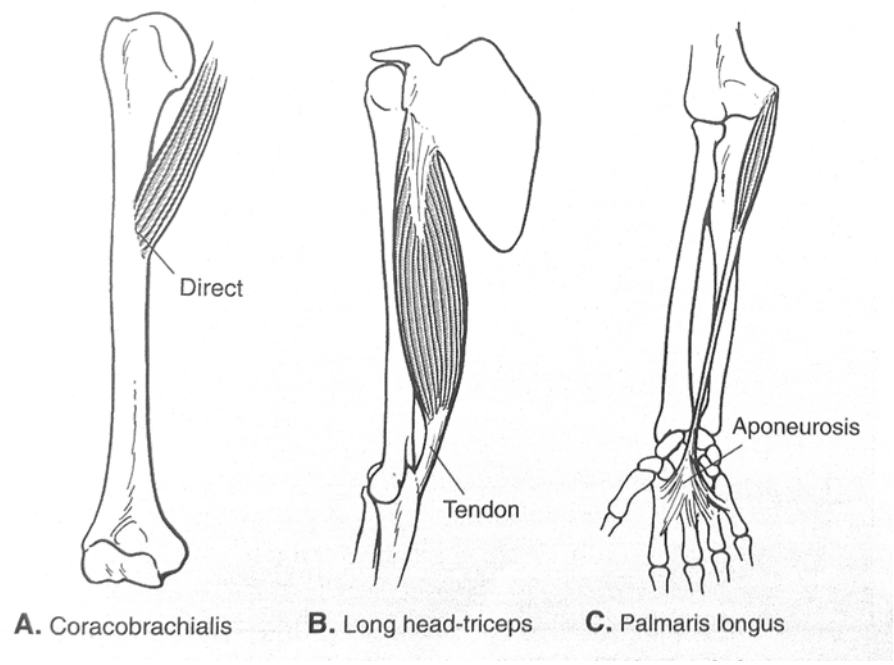


Figure 1.5: Muscle – bone attachments.

(Original figure obtained from Hamill and Knutzen (2006), p73).

Each tendon and aponeurosis has its own mechanical properties; depending on muscle size and purpose, the connective tissue may have different strain patterns or thresholds (Finni, 2006). Tendons and ligaments insert primarily into the outer layer of the periosteum (Buckwalter et al., 1995), however some muscle–bone connections are through fibrous muscle tissue attaching directly into the periosteum. Research suggests that these connections also share molecules in the blood supply, particularly growth factors and myokines, which positively influence the growth and development of both the muscle and bone tissue (Hamrick, 2011). Muscle contraction is the primary source of stimulus for osteogenic response; large peak forces result in an increase in bone mineral density and/or mass, whilst regular low force, high frequency loads also have a positive effect on bone (Hamrick, 2011). The concomitant relationship between muscle and bone is evident in the direct effect of increased muscle mass and strength on BMD (Ahedi et al., 2014). The following section will explore the effect and importance of physical /load bearing activity on both the muscle-bone interaction from childhood into adulthood.

1.4 The Impact of Exercise/Load Bearing activity on skeletal tissue.

The Early Years

Musculoskeletal health and strength is predetermined by genetic factors and growth during childhood. The large advances in bone and muscle growth and development during childhood provide an opportunity to improve musculoskeletal health further, through participation in exercise and regular physical activity (MacKelvie et al., 2002). Khan et al. (2000) reported a number of studies which found that pre-pubertal children who were regularly physically active had higher bone mineral content (BMC) at the total body, femoral neck and lumbar spine, as well as higher bone mineral density (BMD) at the femoral neck, lumbar spine and radius. These children were simply participating in weight bearing activity and play, without any form of prescribed exercise. Similarly, a study by Janz et al. (2010) measured moderate and vigorous everyday activity carried out by children aged 5, 8 and 11 years. The study found a significant ($p<0.05$) improvement of whole body, lumbar spine and hip BMC in children from the age of 5-11yrs who were the most physically active; 8-14% improvement in BMC in boys compared to controls and 6-8% improvement in BMC in girls compared to controls. Participation in moderate and vigorous physical activity optimised their peak bone mass throughout childhood and adolescence. There was also a significant difference ($p<0.05$) between boys and girls activity levels, with boys being more physically active; this difference increased as

age increased (Janz et al., 2010) and may be the key to some sex differences in bone accrual. Additionally, physical activity interventions in school P.E. found moderate impact exercises positively influenced bone health in pre-pubertal children (Khan et al., 2000). MacKelvie et al. (2002) identified the early stages of puberty to be an optimal period for bone gain through exercise, particularly weight bearing and high impact exercise, which can be achieved through a range of sports and activities. Particularly in pre-pubescent girls, the positive effects of high impact sports such as gymnastics have shown significant increases in BMD across skeletal sites, compared to non-gymnasts (Khan et al., 2000).

During adolescence, musculoskeletal mass rapidly increases through an increase in sex hormones at puberty (Bachrach, 2001). Generally, the differences in males and females are significant in relation to muscle mass, strength and bone mineral density (Ettinger et al., 1997; Gallagher et al., 1997). The key to these sex differences occurs during adolescence, where males increase in stature, muscle mass and thereby BMD, due to elevated levels of the sex hormone androgen. As oestrogen is a bone size inhibitor, female adolescents tend to plateau in bone size and BMD at 14-16yrs, which is earlier than males at 15-17yrs (Bachrach et al., 1999). In healthy individuals, adolescent accrual of bone mass can be maintained or improved, with regular exercise and good nutrition (Ortega et al., 2008). The rapid gains in BMD and BMC through exercise during childhood, is less pronounced during puberty (Hind and Burrows, 2007), especially once the growth plates close at maturation, which can be as early as 15 years old in females (Pearson and Lieberman, 2004; Seeman, 2008a). Despite the slowed rate of improvement, the benefits of regular impact or weight

bearing exercise are still very apparent in post-pubertal adolescents, especially with regard to improved bone strength (Khan et al., 2000; Ortega et al., 2005; Ruiz et al., 2006). Regardless of exercise participation, Black ethnic groups have higher BMD across skeletal sites throughout adolescence, than any other ethnic groups, this applies to both males and females (Ortiz et al., 1992; Seeman, 2008a). Subsequently, peak bone mass achieved as adults, is higher in males than females across ethnicities and is higher in Black populations than Caucasian, Asian or Hispanic (Looker et al., 2009). Higher BMD during childhood predicts good BMD in later life (post maturation), therefore the key to preventing age related osteoporosis may simply lie in the encouragement of childhood weight bearing activity (Lanyon and Skerry, 2001; MacKelvie et al., 2002; Hind and Burrows, 2007). Thus, those with higher BMD at key skeletal sites could predictably be higher at all sites of the body, such as the craniofacial skeleton.

Adulthood

During early adulthood, males and females can improve bone and muscle mass through continued regular exercise/loading (Bakker et al., 2003). After peak bone mass, both males and females begin to lose bone mass through an imbalance within the BMUs. It is because of this natural, irreversible resorption of bone in late adulthood that the years preceding peak mass are crucial to securing sufficient bone mass to last throughout life (Heaney et al., 2000). During adulthood the sex difference in skeletal muscle mass is significant in both absolute terms and in

relation to skeletal height (Janssen et al., 2000), with males demonstrating significantly greater amounts of fat free mass. A reduction in relative skeletal mass occurs in the third decade, which coincides with the natural slow progression of bone loss, however decreases in absolute skeletal mass may not occur until as late as the fifth decade (Janssen et al., 2000). The rate of bone and muscle loss is largely controlled by maintaining a healthy active lifestyle with suitable weight bearing exercise interventions (Haskell et al., 2007; Allison et al., 2013). Although exercise seems to induce a positive trend in BMD across skeletal sites, the overall results from intervention trials are non-significant (Kelley et al., 2012). Moreover, adults who experience very low levels of bone mass may need prescribed medication to counteract the effects of conditions such as Osteoporosis (Black et al., 2000). In both cases, intervention cannot improve upon the individual's peak bone mass, but it can slow the rate of loss considerably.

Ageing

Females in particular experience considerable bone loss after menopause that stems from a rapid reduction in oestrogen. This results in an increase in bone resorption, cortical bone thinning and changes to trabecular microarchitecture (Blain et al., 2008). For some individuals this can quickly lead to osteopenia/osteoporosis or related metabolic bone diseases and can be life threatening (Christiansen et al., 1987). This rapid loss is slowed by regular weight bearing exercise and good levels of nutritional supplementation, but cannot be stopped or reversed (Frost, 2001). Kerr

et al. (1996) reported site specific improvements in BMD in post-menopausal women partaking in strength programmes that prioritise high load, low rep exercises. However, women participating in the low load, high rep exercise did not experience the same improvement in BMD, showing that variations in loading may have different effects on BMD (Kerr et al., 1996). Even into old age, the positive effects of exercise and good nutrition can be seen in bone and muscle health (Allison et al., 2013), functionality and decreased risk of chronic disease or fracture due to falling. During ageing, loss of bone mass is more rapid in Caucasian cohorts than Asian (Finkelstein et al., 2002), which brings the BMD to a similar level. Black populations maintain higher BMD than all others throughout, but still experience bone loss (Looker et al., 2009). With regard to muscle, there is a greater loss of strength than mass in both sexes with age, suggesting a loss of muscle quality rather than size, regardless of activity (Goodpaster et al., 2006). Both males and females experience a reduction in appendicular skeletal muscle mass (Gallagher et al., 1997) however, Caucasian males experience a greater loss than women (Goodpaster et al., 2006) and the rate of loss increases with age (Hughes et al., 2001). Black cohorts experience a greater loss in muscle strength than Caucasians in both males and females (Goodpaster et al., 2006) despite a stable reduction in BMD. Salkeld et al. (2000) found that women over the age of 75 years experienced considerably reduced quality of life due to fear of falling and subsequent hip fracture. In extreme cases, medical intervention can only improve BMD at key skeletal sites by 1-4% (McClung et al., 1998), which may reduce the risk of fracture if falls occur (Black et al., 2000). Musculoskeletal diseases, such as osteoporosis, cannot be improved through

exercise, but exercise can be used as a preventative measure throughout life and as a medium through which musculoskeletal health may be maintained. Ireland et al. (2014) measured muscle size, bone mineral content (BMC) and bone area in elite veteran tennis players, they reported that bone area and periosteal circumference asymmetries were greater ($p<0.01$) in athletes that started playing during childhood/adolescence and continued throughout life, than those that started during adulthood. Furthermore, Wilks et al. (2009) measured the tibia of 106 sprinters, 52 middle distance runners, 93 long distance runners, 49 race walkers competing in master championships and 75 age matched, non-athletic controls (aged 35-94yrs). The study reported that all athletes had higher tibial BMC and cortical area than controls, and that male and female sprinters had higher (15% and 18% respectively) epiphyseal BMD than controls ($p<0.001$). In relation to key skeletal sites, research has shown increased BMD through exercise/loading. Thus, the loading effect on bone density in the mandible may be achieved through higher bite forces and muscle activity during mastication.

1.5. The Craniofacial Skeleton

Craniofacial Development

In terms of musculoskeletal growth, the craniofacial system develops in much the same way as the rest of the body. At birth the skull is unformed; the skull plates are not fused together and the bone has very low mineralisation (Currey, 2002). In addition, the cranial bones are of a uniform, porous material at birth, which develops into a sandwich of cortical and cancellous bone structure during growth (Currey, 2002). The craniofacial system comprises of many flat or irregular bones, which do not have epiphyseal growth plates like those found in long bones (Buckwalter et al., 1995). Instead, the craniofacial bones most commonly change size and shape at varying growth rates at the periosteal surface, which is facilitated by the interdigitations or sutures between bones (Van Spronsen et al., 1997; O'Higgins et al., 2012; Maloul et al., 2013). The sutures between the craniofacial bones remain key areas for development and expansion of the skull during growth (Lee and Moon, 2012). The craniofacial bones and sutures complete the growth phase during adolescence, but some facial bones and sutures continue to change during late adolescence and into early adulthood (Nahhas et al., 2014). The growth and development of the craniofacial skeleton varies considerably between individuals and over time. It has been reported that the onset of accelerated growth due to puberty can occur anywhere between 7-10yrs, the peak velocity of pubertal growth

can occur anywhere between onset and 14yrs and the age of cessation may occur anywhere up to 20yrs (Nahhas et al., 2014). Furthermore, the time frames are not only different for individuals but they also vary between sites on the craniofacial skeleton. Nahhas et al. (2014) reported that the cranial base length began accelerated growth later in childhood (aged 8.5-9yrs) than the length of the mandible and maxilla (7-8yrs), but also reached cessation earlier (15-18yrs compared to 17-20yrs). This indicated that changes to the jaw bones are still occurring into early adulthood, and may affect the individuals' bone strength at the mandible and maxilla. Bones, muscles and dentition grow at different rates and over different periods, which can be explained by genetics, hormones and mechanical influences from masticatory forces and/or muscle pull (Kiliaridis, 1995; Van Erum et al., 1997; Young et al., 2000; O'Higgins et al., 2012). In general, the craniofacial bones and muscles increase in size and change shape rapidly up to 12 months of age and then steadily develop throughout childhood (Le Révérend et al., 2014). During the first three years, the eruption of dentition occurs in stages beginning with the incisors, the 1st molars, the canines and finally the 2nd molars. This constant state of fluctuation negatively influences jaw muscle activity and bite force, until a full set of dentition is achieved (Palinkas et al., 2010; Le Révérend et al., 2014). During this time, there are many changes to the soft tissues of the face and the facial muscles, in particular the thickness and efficiency of contraction of the jaw elevator muscles, as children develop the ability to chew hard foods (Green et al., 1997; Le Révérend et al., 2014). In young children, the chewing motion remains purely vertical even as late as three years of age, until children develop the ability to rotate the jaw using lateral

movements (Le Révérend et al., 2014); this is thought to develop in accordance with craniofacial structure and stability through the interaction of the muscle–bone interface (O'Higgins et al., 2012). A study by Larsson (1997) showed that hard foods are key to developing masticatory function and thereby, the strength of the craniofacial musculoskeletal system. Larsson (1997) compared Swedish and Norwegian children who experienced different weaning habits as babies; the cohort that were given hard bread to chew on from an early age developed wider facial dimensions and fewer instances of occlusal crossbite, than those fed soft foods. Similar to the rest of the skeleton, much advancement in craniofacial strength, structure and function is developed during childhood and adolescence, partly due to genetic and endocrine factors, but largely due to mechanical loading. Despite this, further advancement and changes may be seen throughout adulthood, and certainly changes occur in old age due to reduced muscle force and edentulous participants (Raadsheer et al., 1999; Dechow et al., 2010).

As previously described, from a whole body perspective the musculoskeletal system is largely maintained and strengthened through loading, with particular emphasis on weight or load bearing exercise to produce increased bone mineral density (Frost, 2003). The craniofacial skeleton only experiences low forces unlike those imposed onto the lower extremities, with the exception of those experienced during trauma. Forces applied to the craniofacial skeleton may be produced by a change in pressure inside the skull during growth, due to the expansion of the brain (O'Higgins et al., 2012). Most commonly, forces are produced by muscle contractions during movement of the head or jaw (Kiliaridis et al., 1995; Van Spronsen et al., 1997).

Some theories and research based evidence presented in recent studies regularly exclude the skull as an exception to the mechanical loading rules (Frost, 2003), despite findings that attribute sutural growth to a combination of genetic pathways and mechanical stimuli (Mao, 2002; Maloul et al., 2013). This suggests that the craniofacial skeletal system must exhibit remodelling thresholds which are low enough to allow bone to be maintained by low strains (Dechow et al., 2010). In addition, the craniofacial skeleton can accommodate relatively high strains from masticatory loading (Van Eijden, 2000) and requires these strains for accelerated osteogenic response and increases to cortical thickness (Gröning et al., 2013). Research exploring the differences in craniofacial morphology has found jaw elevator muscle size to have a positive influence on facial structure (Raadsheer et al., 1999; Suda et al., 2002), whilst jaw elevator muscle pull has been found to significantly influence facial morphology, corresponding to muscle attachment sites on the mandible (Van Spronsen et al., 1997). Furthermore, Kiliaridis (1995) reported that the elevator muscles affect the transversal and vertical dimensions of the face; an increase in loading causes an increase in sutural growth due to repeated strains on the bone. This in turn can affect the development, size and shape of the dental arch, which may allow for further improvements of occlusal and bite force capabilities (Raadsheer et al., 1999; Regalo et al., 2008). It appears the craniofacial skeleton is both maintained and improved through a muscle-bone-dentition cycle: higher bite force leads to increased muscle strain (Gröning et al., 2013), which produces an osteogenic response (Van Spronsen et al., 1997) and through improved skeletal structure, bite force capabilities are increased (Raadsheer et al., 1999).

The male skeleton tends to grow larger from the periosteal surface than the female skeleton, which results in greater volumetric bone mineral density and thereby stronger bones (Seeman, 2008a). This may also be true of the craniofacial skeleton, as circulating growth hormones have been found to develop the size and angular relationship of the facial bones, as well as the incisal dentition in mice (Ramirez-Yañez et al., 2005). Similarly, growth hormone and testosterone therapy in children lead to improved craniofacial growth much like the rest of the skeleton (Van Erum et al., 1997; Verdonck et al., 1999). Craniofacial morphology is most commonly investigated using cephalometric dimensions from lateral radiographs. Much of the craniofacial size differences between males and females stems from adolescence, where males continue to grow for longer, resulting in longer or larger facial dimensions at maturity. For example, Nahhas et al. (2014) reported age of cessation for the mandibular length as 17yrs in females, 20yrs in males, for the maxilla length 16yrs in females, 20yrs in males and for the cranial base 15yrs in females and 18yrs in males. Sexual dimorphism in cephalometric dimensions is apparent across a number of ethnicities, including Caucasian, European, Japanese, Chinese and Black cohorts. Findings suggest that males have larger craniofacial lengths than females, for example Miyajima et al. (1996) reported a significantly greater ($p<0.01$) mean mandibular length (125.5±5.1mm) in males than (118.8±4.7mm) females, but differences in angular measurements vary and are less often significant (Miyajima et al., 1996; Gu et al., 2011). Furthermore, Japanese females have been found to

exhibit a steeper mandibular plane angle in comparison to their male counterparts (Miyajima et al., 1996). Greater mean midfacial height and mandibular length have been found in males (74.9mm Chinese, 71.7mm Caucasian), compared to females (69.4mm Chinese, 65.0mm Caucasian), of both Caucasian and Chinese ethnicity (Gu et al., 2011). Franklin et al. (2008) examined sexual dimorphism in Black South Africans for use in forensic science; all nine measurements taken of the mandible were significantly different between males and females, but coronoid height (58.5±4.7mm male, 52.46±3.66mm females), ramus height (56.66±4.94mm males, 50.96±3.66mm females) and mandibular length (120.89±4.65mm males, 114.85±4.73mm females) were the greatest predictors. Despite a strong trend towards greater dimensions in males, some research suggests that females have a greater number of remodelling agents, including osteocytes and lacunae, on the parietal surface in the skull (Torres-Lagares et al., 2010). Although this may indicate a more adaptable area of bone in females, the remodelling activity was not significant between sexes; Torres-Lagares et al. (2010) reported that remodelling remained low and stable in the parietal bone regardless of sex. Furthermore, Torres-Lagares et al. (2010) reported a similar rate of decline in parietal remodelling with age, between males and females. These findings are in contrast to Doual et al. (1997) who reported a more pronounced change in cephalometric dimensions, particularly posterior facial height and cervical vertebral height loss, amongst women during the menopause, compared to men of a similar age. Doual et al. (1997) reported accelerated loss of bone structural height at the superior facial structure and cervical vertebral column, as opposed to changes in the cranium as reported by Torres-

Lagares et al. (2010), which may indicate different rates of growth, remodelling and age-related change (O'Higgins et al., 2012).

The process of ageing greatly affects the development of the human skeleton, ultimately resulting in a halt in growth and a gradual reduction in material properties such as bone mineral density and cortical thickness (Seeman, 2004; Blain et al., 2008). These changes are slow and stable throughout adulthood, with large or dramatic changes only occurring through immobility, disease or menopause in females (Christiansen et al., 1987; Frost, 2001; Blain et al., 2008). Conversely, the craniofacial skeleton appears to continue to grow throughout adulthood and even into old age (Israel, 1973; Doual et al., 1997; West and McNamara Jr, 1999). Research suggests that the skull continues to deposit new bone, albeit slowly, so that the cranial bones continue to thicken over time (Doual et al., 1997), even at the endocranial surface (Israel, 1973). The craniofacial growth is acknowledged as symmetrical, or at least uniform, across craniofacial bones except for the skull thicknesses and the frontal sinuses which grow at different rates (Israel, 1973). West and McNamara Jr (1999), investigated the changes in the craniofacial skeleton from adolescence (aged 15-18yrs) to mid-adulthood (defined as 45-50yrs) in a large cohort of males and females. The study reported continued growth of skeletal, soft tissue and dentoalveolar measurements throughout maturation. West and McNamara Jr (1999) reported angular measurements didn't significantly change in males or females over time. From adolescence to early adulthood females continued to change angularly, exhibiting a posterior rotation of the mandible, but this did not continue into mid adulthood and was not was not significant. Conversely, males

experienced a small anterior rotation of the mandible, which was also not significant (West and McNamara Jr, 1999). Linear cephalometric measurements including midfacial height, mandibular length and anterior facial height increased significantly over time, which also coincided with continued tooth eruption (West and McNamara Jr, 1999). Similar changes to the maxilla bone measurements were not found by Israel (1973), but changes to the material properties of the maxilla were reported by Dechow et al. (2010). Doual et al. (1997) also reported mixed findings from a large cohort of males and females, concerning the craniofacial structure, superior facial structure, mandible and the cervical vertebrae. The changes in craniofacial bone lengths over time could affect the loading patterns and thereby site specific changes in BMD may be detected. Of the facial structure measurements, most notably the posterior facial height increased with age, similar to other findings (West and McNamara Jr, 1999) and the maxillary sinus demonstrated no age related changes, unlike other studies (Israel, 1973). As with many other ageing effects on the skeleton, Doual et al. (1997) reported numerous and apparent changes in craniofacial morphology occurred around the age of fifty, with the greatest change occurring in females post menopause. Furthermore, Doual et al. (1997) suggested that the greatest modifications in bone structure occurred in bones of membranous origin (such as the frontal, parietal, nasal, maxilla, zygoma and mandible), whereas, bones of endochondral origin (such as the nasal septum, occipital bone, skull base and mandibular condyle) remain more stable throughout life. As well as changes in length and angular rotation, the ageing process may affect the material properties of the craniofacial skeleton, which may have greater consequences for skeletal health

and strength, than dimensional changes (Dechow et al., 2010). Edentulous skulls have been found to demonstrate thinner cortical bone across all regions of the face as well as reduced BMD, elastic moduli and shear moduli in the maxillae. The loss of masticatory function due to ageing, dentition or changes to craniofacial morphology may result in regional changes to bone remodelling, and a reduction in biomechanical properties such as bone mineral density (Horner et al., 1996; Dechow et al., 2010).

The effects of ethnicity on cephalometric differences has been explored between Japanese, European-American, Caucasian, Chinese, African-American and Black populations, amongst others (Drummond, 1968; Connor and Moshiri, 1985; Miyajima et al., 1996; Gu et al., 2011). Comparison of Caucasian with Asian populations find that Japanese and Chinese people have a smaller mid facial height and shorter mandible length in men and women (Gu et al., 2011), but not in Japanese women (Miyajima et al., 1996). Miyajima et al. (1996) also reported larger vertical facial axis with significant downward growth in the Japanese population, whereas Gu et al. (2011) also reported a retrusive chin in Chinese women and a significantly steeper mandibular plane in both male and female Chinese. Comparison of white and black populations show substantial dentofacial differences (Kowalski et al., 1974) with the most common (Drummond, 1968; Connor and Moshiri, 1985; Kowalski et al., 1974) and in some cases the strongest differences (Bacon et al., 1983) reported to be the position and angle of the incisors. Amongst other measurements, Drummond (1968) noted differences in the maxilla relative to the mandible and to the anterior cranial base, resulting in maxillary prognathism (also

known as protrusion) in the black cohort, which was also reported by Bacon et al. (1983), Kowalski et al., (1974) and Connor and Moshiri, (1985). Furthermore Black populations exhibit a greater mandibular length (Connor and Moshiri, 1985), a steeper mandibular plane (Drummond, 1968) and several labial and soft tissue differences (Bacon et al., 1983; Connor and Moshiri, 1985) to Caucasians.

Regardless of the effects of sex, age and ethnicity, the differences in craniofacial morphology can affect the functionality of the masticatory system and even the material properties of the craniofacial bones. Due to the vast number of cephalometric variables, it can be difficult to draw conclusions within or between groups, but research has found craniofacial morphology affects bite force (Braun et al., 1995b), muscle activity (Gomes et al., 2010) and bone thickness (Kohakura et al., 1997). Bite force in particular is a strong predictor of masticatory function, Raadsheer et al. (1999) reported that craniofacial morphology explained 58% of the variance within bite force in a Caucasian cohort, regardless of sex. Furthermore, Braun et al. (1995b) reported that maximum bite force was affected by mandibular plane angle as well as posterior facial height, this was corroborated by Van Spronsen et al. (1997) who linked these differences in morphology to the spatial orientation of the jaw elevator muscles. In terms of mechanical levers, the length and angle of the mandible will affect the movement and muscular force needed to open and close the jaw (Van Spronsen et al., 1997; Van Eijden, 2000). Therefore, differences in craniofacial morphology can affect masticatory function, occlusal contact area, bite force and masticatory muscle activity (Gomes et al., 2010; Custodio et al., 2011). With regard to masticatory function, some research suggests that craniofacial

morphology can cause excessive tooth wear in adulthood (Almond et al., 1999) and this in turn can increase the stress placed on the craniofacial bones (Kiliaridis et al., 1995) In addition, Hara et al. (2010) suggested craniofacial morphology affected the fatigability of the masseter muscle, in a Japanese cohort. Regardless of the sex, age or ethnicity of the sample group, craniofacial differences can have a subsequent effect on the growth patterns of other facial dimensions (Costa et al., 2012), as well as a potential effect on the material properties of bone (Kohakura et al., 1997).

1.5.1 Bite force

Bite force measurements are the best non-invasive indicator of peak forces acting on the craniofacial skeleton. Peak forces and strains induce osteogenic responses within regions of loaded bone tissue, which cause increases in BMD, cortical bone thickness or structural changes at loaded sites (Frost, 2004), but loading within the craniofacial skeleton is not as well understood. Differences between sexs, age categories and ethnicities affect the growth and development of the craniofacial skeletal system, in turn these may affect the functional capability of the jaw in producing maximal forces for mastication (Custodio et al., 2011; Nahhas et al., 2014). The following section will review bite force studies that compared findings across sexs, age categories and ethnicities. It is pertinent to identify here, that different bite force devices and manufacturing specifications of equipment may affect human bite force mechanics (Paphangkorakit and Osborn, 1997; Rues et al., 2008). This is one contributor to the variation in results reported for adult maximal bite force. However, the differences in equipment, the merits and issues concerning key types of bite force device, will be discussed in Chapter2, Section 2.3.

Amongst children and adolescents bite forces have been reported to range from 50N to 600N (RoldÁn et al., 2009) depending on age, sex and position of biting device. In a study of the reliability of bite force measurements in children aged 5-14yrs, RoldÁn et al. (2009) reported that age accounted for 50-71% of the between subject bite force variance. Furthermore, the bite force differences between children are often reliant on their dental stage (Le Révérend et al., 2014). This is supported by Pereira

et al. (2007) who reported significantly lower molar bite forces in boys and girls with mixed dentition (345.7-356.9N), compared with boys with permanent dentition (387.4±27.6N). These findings may be due to the 2-4 year age gap, which encompassed the onset of puberty, and the difference in dental maturation (Pereira et al., 2007; Le Révérend et al., 2014). Moreover, Varga et al. (2001) reported the absence of sex differences in bite force at 15 years of age (early puberty) compared to significantly higher bite force in males at 18yrs (late puberty/cessation), in a Caucasian cohort. They also reported significantly higher bite force in 18 year old males (777.7±78.7N) compared to 15 year old males (522.3±181.7N) but no age difference in females, suggesting a large advancement in bite force in males towards the end of puberty, which may be linked to prolonged skeletal growth caused by male sex hormones. In contrast, from a sample group of 8-68yrs old males and females, Bakke et al. (1990) found a steady increase in bite force in both sexs up to the age of 25, which culminated in an average bite force of 572N. After 25yrs, female bite force began to decline and male bite force plateaued until 45yrs, when it began to decline. Similarly, Palinkas et al. (2010) measured a cohort of males and females aged 7-80yrs, subdivided into 5 age groups. They reported that the youngest group (aged 7-12yrs) and the oldest group (aged 61-80yrs) had significantly lower bite force (185±30N males, 163±30N females age 7-12yrs) than the other 3 age groups (405±30N males, 280±31 females aged 13-20yrs), which mainly comprised of adults with some adolescents with permanent dentition (Palinkas et al., 2010).

Bakke et al. (1990) also reported that sexual dimorphism was not significant until 30 years of age, where it remained until 60 years of age. Conversely, in a study with a

larger cohort of males and females, Palinkas et al. (2010) found sex differences in maximum bite force from the age of 13 – 80yrs. This onset of sexual dimorphism occurred much earlier than in studies that focussed on childhood and adolescent bite force only (Pereira et al., 2007; Varga et al., 2011). Studies that measured maximal bite force in adult cohorts have reported mixed results with regard to sex differences. Significant differences in maximal bilateral bite force have been found between young adult males and females in several studies (Braun et al., 1995a; Shinogaya et al., 2001; Van Der Bilt et al., 2008). However, similar studies have also reported no significant differences between young adult males and females (Paphangkorakit and Osborn, 1997; Thompson et al., 2001; Lepley et al., 2011). These differences may simply be due to the size and variation within each sample group, for example Braun et al. (1995a) used a large but uneven sample of 86 males and 56 females, which resulted in a significantly different average maximal bite force of 814N for males and 615N for females. Conversely, Thompson et al. (2001) reported no significant difference between sexs in a sample of 15 males and 15 females. Kiliaridis et al. (1995) measured bite force in a sample of males and females in their early- to mid-adulthood, exhibiting worn dentition. The study reported no significant sex difference during maximal biting, but did find males used a significantly higher force during normal 'chewing'. Furthermore, Ferrario et al. (2004) measured bite force at each individual tooth from the central incisors to the 2nd molars, bilaterally. The study reported that young adult males demonstrated significantly higher force at each tooth than young adult females. In general, sexual dimorphism is acknowledged and expected within bite force measurements

conducted on adults (Bakke, 2006; Palinkas et al., 2010), until 60 years of age or older when differences seem to reduce (Bakke et al., 1990; Motegi et al., 2009) either due to the onset of ageing or poor dental condition (Tortopidis et al., 1999; Caloss et al., 2011).

The effect of ethnicity is under explored within bite force studies. The vast majority of subjects measured are Caucasian (Ferrario et al., 2004; Varga et al., 2011) or their ethnicity is unreported (Braun et al., 1995a Kiliaridis et al., 1995; Paphangkorakit and Osborn, 1997; Van Der Bilt et al., 2008; Lepley et al., 2011). Regalo et al. (2008) measured maximal incisor and molar bite forces between Caucasian and indigenous young adults of Brazilian nationality. They reported significant differences between ethnicities in incisal bite force, (206±24N Indigenous males, 150±18N Caucasian males, 140±20N Indigenous females, 93±15N Caucasian females) but not molar bite force (502±47N Indigenous males, 484±53N Caucasian males, 272±34N Indigenous females, 288±50N Caucasian females). Regalo et al. (2008) attributed these differences to the eating habits of the indigenous population. Shinogaya et al. (2001) compared young adult Japanese females with Danish females, they reported a significantly higher average pressure at the canines, pre-molars and molars (37.6±3.5MPa Danish, 42.3±3.8MPa Japanese, $p<0.01$) in the Japanese cohort, these differences were attributable to facial morphology and occlusal contact area (Shinogaya et al., 2001).

The craniofacial skeleton is capable of continued remodelling throughout life (Doual et al., 1997; West and McNamara Jr, 1999), as well as generating maximal masticatory forces ranging from 200N to 800N during adulthood (Tortopidis et al.,

1998; Sondang et al., 2003). Therefore, it is pertinent to expect that peak forces experienced through mastication may improve site specific BMD in the facial skeleton. Furthermore, the relationship between bite force, muscle activity and bone mineral density of the facial bones is only partly explored by existing research studies (Kohakura et al., 1997; Gomes et al., 2010; Custodio et al., 2011), and is (to the authors knowledge) completely unexplored in some ethnicities, particularly African - Caribbean.

Aims and Objectives

The aim of the present research study is to investigate the relationship between bite force, masticatory muscle activity and bone mineral density. In addition, the study will explore the effect of sex, age, ethnicity and facial dimensions on the jaw elevator muscle, mandibular bone and bite force relationship.

To accomplish these aims, the objectives of the study are -

(i) To measure the muscular activity in the jaw elevator muscles.
(ii) To measure maximal and sub-maximal bite force.
(iii) To measure BMD at hip, lumbar spine and two sites on the mandible in order to identify relationships between BMD values at different sites.
(iv) To measure facial dimensions and investigate the interaction with bite force, muscle activity and mandibular BMD.

This research study will measure a young adult cohort of Caucasian British males and females who will form a healthy baseline for comparison between young and older adults. The younger cohort results may be beneficial for identification of those at risk of facial sports injury, with regard to how facial protection within sport may be beneficial for those with lower facial BMD in this cohort. This study will also measure a 50+yrs cohort of males and females, which will provide an insight into the changes that occur in an older sample of males and females who are free of metabolic bone diseases and will make comparisons to studies that have evaluated BMD in osteoporosis studies, specifically highlighting the mandible as a key site. This may also reflect the level of risk of facial fracture experienced by healthy active over 50yrs individuals. The final cohort of young adult African Caribbean British males will

provide an insight into the facial bone differences in an ethnic group, which is already recognised for its high bone mineral density at loaded skeletal sites. It will allow comparisons in relation to facial sports injury, which is an under researched area particularly with regard to differences between ethnicities.

Chapter 2: Methods

The following chapter details the experimental testing procedures conducted throughout this research study. It also evaluates the design protocol, which encompasses the development and choice of the appropriate test procedure for measuring facial muscle activity, as well as the appropriate equipment for measuring bite force. Furthermore, it investigates whether facial electromyography (EMG) can be successfully normalised to a sub-maximal bite force. The purpose of the normalisation method was to allow for all cohorts to be examined within this study, whether they exhibited restricted movement/bite force ability (such as the elderly) or not. Finally, this chapter aims to justify the use of certain measurement techniques used throughout the thesis, in particular the use of Dual Energy X-ray Absorptiometry (DXA) and facial dimension analysis using photographs rather than radiographs.

2.1 Measurement of Muscle Activity.

Electromyography is the process by which electrical signals are detected using electrodes (Hamill and Knutzen, 2006). Electromyograms (EMGs) detect the presence of electrical activity in the sarcolemma, known as the action potential. The action potential propagates from the Innervation Zone (IZ), which is the site of the motor point within the muscle fibres and is commonly found in the muscle belly (Mesin et al., 2009). The action potential signal runs in both directions from the IZ along the muscle fibres. EMGs also infer information about the timing, magnitude and frequency of the signal content. During muscular contractions, the muscle activity and muscle force are correlated (Disselhorst-Klug et al., 2009), thereby an increase in muscle force results in a measured increase in the amplitude of the EMG (Hamill and Kutzen, 2006). The relationship between muscle force and EMG differs between muscles, magnitude of contraction and even the standard to which the muscle has been trained. In general, higher EMG amplitudes may be explained by either an increase in motor unit (MU) recruitment or increased firing rates, or by an increase in both (Madeleine et al., 2001). However, the force/EMG amplitude relationship isn't exact and may contain areas of both linear and curvilinear association (Disselhorst-Klug et al., 2009). Lawrence and De Luca (1981) showed variation in the results for muscles in the upper extremity, the biceps brachii and the deltoid exhibited a curvilinear signal-force relationship, whilst the first dorsal interosseous in the hand showed a linear relationship. They found no group effect on the signal-force relationship during isometric contractions. Furthermore, the

force/amplitude relationship during concentric and eccentric contractions has shown muscle activity to increase in concentric and decrease in eccentric contraction, compared to static isometric contraction, at the same force values (Disselhorst-Klug et al., 2009). Furthermore, Merletti and Parker (2004) reported that MU recruitment occurs at greater force in eccentric contraction compared to concentric. This restricts the predictability of force values from muscle activity, to require complex algorithms. Madeleine et al. (2001) measured the concentric, isometric and eccentric EMG signal-force relationship in the first dorsal interosseous muscle at 0, 25, 50 and 75% of MVC. Their findings showed a significant ($p<0.05$) difference in EMG root mean square (rms) values from 0-50%, in the concentric and isometric contractions and from 0-75%, in the eccentric contractions. The overall findings suggest a curvilinear relationship but the significant differences found in lower %MVC suggest a linear relationship below 50% MVC. These findings are concurrent with Perry and Bekey (1981) who found a linear relationship at low to moderate force levels but a non-linear relationship at moderate to high force levels.

Study Practical Considerations

EMG electrodes may be either intramuscular needle (fine wire) or surface electrodes; the former is inserted into the muscle tissue and is capable of detecting small numbers of- or individual MUs (Merletti and Parker, 2004). The latter are placed over the skin covering the muscle, it detects many MUs in one signal and has always been favoured in research and clinical investigations for its non-invasive

qualities (Basmajian, 1962; Castroflorio et al., 2005; Suvinen and Kemppainen, 2007; Mesin, et al., 2009). Therefore, sEMG will form the majority of the following discussion.

Surface EMG (sEMG) recordings are commonly derived from the difference in electrical potential detected by two electrodes, termed bipolar electrodes (Hamill and Knutzen, 2006). Additionally, linear or multi-linear arrays are also used, which cover a larger area of the muscle and provide several differential signal outputs. This can be used prior to testing to identify the position on the muscle that offers the best signal, thereby offering improved accuracy and repeatability of electrode placement for sEMG data collection (Farina et al., 2004; Kendell et al., 2012). Surface EMG electrodes can vary in size, shape and material, there is no 'gold standard' electrode, but some are better suited to certain tasks. Electrode dimensions range from $1mm^2$ to several cm^2 and circular electrodes as large as 8-10mm in diameter (Castroflorio et al., 2008). For smaller muscles, such as facial muscles, electrode sizes of <10mm in length or diameter provide more selectivity within the muscle, and electrodes >10mm with greater inter-electrode distance provide monitoring for larger muscles (Cram et al., 1998). During data collection, a third electrode is placed on a non-muscular neutral landmark to act as a reference.

Signal Quality

Factors that may affect the signal strength and quality of the EMG recording, can be categorized as either biological variables or process variables. Examples (by no means exhaustive) of biological variables include: muscle fibre type, size, orientation, position of the IZ, MU firing rate and recruitment, subcutaneous fat and distance of the muscle from the surface. Examples (by no means exhaustive) of process variables include: Electrode size, shape, position, and spacing, electrode to skin interface, and signal conditioning (Farina et al., 2004; Hamill and Knutzen, 2006; Enoka, 2008). Largely, the biological variables that affect EMG signal quality cannot be altered but with a careful protocol, the process variables can be optimised to improve the signal (Castroflorio et al., 2005; Mesin et al., 2009). Electrodes should not be placed over or relatively close to tendinous areas (Enkoa, 2008) as the action potential becomes weaker towards the ends of the muscle fibres which would result in a weak- or loss of signal. This may manifest as a problem in larger muscles performing gross motor tasks, which cause movement of the muscle under the surface of the skin due to concentric contraction (Finni, 2006). Additionally, if the electrodes are placed either side of the IZ, they will record symmetrical or near symmetrical potentials propagating in opposite directions, which would result in very small signals and an incorrect representation of the muscle activity. Other than amplification, the differential signal recording is dependent on a number of affecting factors; the influence of the IZ, cross talk from neighbouring muscles and unwanted signal content. Larger limb muscles tend to have concentrated IZs, which are detectable

and avoidable during sEMG electrode placement (Kendell et al., 2012). Some smaller, more irregular muscles such as the facial muscles have scattered IZs which makes placement less reliable (Castroflorio et al., 2005). The scatter of IZs may be different in different muscles and for the same muscle in different people, this may partly explain the differences in findings between studies and supports the notion that researchers should check for the location of the IZ before electrode placement (Mesin et al., 2009).

Crosstalk occurs when the electrodes detect the signals of the muscle fibres from a neighbouring muscle, along with the propagation signals from the intended muscle (Enoka, 2008). The relative contribution of cross talk increases with fat thickness and electrode placement over the tendon, but is not influenced by electrode distance. It may also be increased by closely positioned or overlapping muscles such as those found in the face (Suvinen and Kemppainen, 2007). Cross talk is hard to remove and most sEMG signals will inevitably contain some amount of cross talk (Criswell, 2010). Even with a strict experimental procedure, the issue of crosstalk from nearby muscles may interfere with the signal from the intended muscles. De Luca and Merletti (1988) recorded EMG signals from the tibialis anterior, peroneous brevis and the soleus during activation of the tibialis anterior. They reported as much as 16-17% of muscle activity in the lower leg as crosstalk from neighbouring muscles. Solomonow et al. (1994) examined the medial and lateral gastrocnemius and tibialis anterior of the lower limbs of cats. The study reported sEMG crosstalk levels less than 5%, however the amount of crosstalk was negatively influenced by the amount of subcutaneous fat beneath the electrode, which is known to have a filtering effect

on the signal (Perry and Bekey, 1981; Merletti et al., 2002). Where functional tests may be performed to isolate individual muscles for signal comparison during a multi-muscle movement, authors may be able to estimate the quantity of crosstalk in a signal (Burden, 2007).

The amplifier should undertake the removal of unwanted signal content, such as environmental electrical noise, using a filter. Filters may be either low pass, high pass, band pass or band stop (Enoka, 2008). Low pass eliminates high frequencies, high pass eliminates low frequencies, band pass eliminates frequencies above or below a specified band and band stop eliminates just one specified frequency. However, after this process an EMG signal may still contain several 'unwanted' elements that need to be eliminated; other than crosstalk, these may include environmental noise, biological noise and movement artefacts (Enoka, 2008). Typically, raw EMGs are rectified and smoothed before data analysis. Rectification requires calculating the absolute value of the signal by inverting the negative values to create positive values. The rectified EMG is digitally filtered to remove specific frequencies within the signal, this most commonly has a low pass filter effect, which produces a smoothed signal comprised of averaged data points (Criswell, 2010). The type of signal processing should be tailored to the task and the subsequent analysis. The next section will detail the facial electromyography case study, a preliminary experiment intended to identify the specific requirements of a jaw elevator muscle EMG protocol.

2.2 Facial Electromyography Protocol: Muscle Selection, Electrode Position, and Facial Expression.

Introduction

Electromyography has been utilised as a clinical tool in dentistry and craniofacial research (Lund and Widmer, 1989; Suda et al., 2002; Gomes et al., 2010; Linsen et al., 2013). Craniofacial studies that include EMG analysis have commonly used small (2-10mm) electrode sizes (Siéssere et al., 2009), which are often made from Silver/Silver Chloride (Ag/AgCl) alloy. The masseter and the anterior portion of the temporalis, also referred to as jaw elevator muscles, are the most commonly used jaw muscles in bite force studies (Vianna-Lara et al., 2009). The elevator muscles contract during biting, clenching or grinding of the dentition (Yoshimi, 2009). The masseter and anterior temporalis are the largest of these (Palinkas et al., 2010) and are superficial, which makes them easily compatible when using the sEMG technique (Bakke, 1993). Despite the extensive use of the masseter and anterior temporalis in the existing literature, researchers still use a variety of approaches for measuring the muscle activity, in terms of electrode size, shape and placement.

With regard to techniques for positioning EMG electrodes, some studies simply recommend positioning the bars over the muscle belly, usually through palpation (Burnett et al., 2000; Ferrario et al., 2004; Vianna-Lara 2004; Siéssere et al., 2009). Conversely, Castroflorio et al. (2005) investigated the electrode location and inter-electrode distance on facial EMG signals in young healthy adults. They concluded

that EMG signals are very sensitive to electrode displacement, even as little at 2.5mm distance, due to scattered innervation zones and short muscle fibre lengths in the elevator muscles. However, they found that the high sensitivity to electrode location reduced with inter-electrode distances of 10-15mm. Each individual could have different optimum electrode locations, which would require prior investigation and may not be feasible in some research studies due to time and equipment constraints. Thus care should be taken to enforce a strict placement protocol to reduce total variability (Castroflorio et al., 2008).

Craniofacial EMG Crosstalk

The facial muscles lie in very close proximity, often running parallel or overlapping with one another (Suvinen and Kemppainen, 2007) this makes the possibility of recording activity from only one facial muscle very difficult (Lapatki et al., 2003). Additionally, many muscles contribute to the jaw closing action, the most prolific of which include the superficial and deep masseter, anterior and posterior temporalis, as well as the medial and superior portion of the lateral pterygoids (Gray, 2010), none of which can be isolated through functional tests. As a possible solution to the problem of cross talk, intramuscular electrodes have been used to measure facial muscle activity (Rues et al., 2008), as they are superior to surface electrodes with regards to precision. Additionally, they are less likely to obstruct fine facial movements because they are small and they do not require as much taping to hold them in place in the skin. However, the process is invasive for facial nerves and

participants may experience a great deal of discomfort (Schumann et al., 2010). The present study does not warrant the use of invasive techniques for measuring muscle activity, therefore the issue of crosstalk will have to be addressed through a strict placement protocol.

Craniofacial EMG Reliability

The reliability of electrical potential recordings have been found to be high when studied over short periods of time (< 1 hour), when the reliability has been studied across longer periods of time, or the electrodes have been re-positioned, the reliability has decreased (Cram et al., 1998). Within craniofacial research, Saifuddin et al. (2001) found dynamic movements of the jaw elevator muscles to produce reliable between-session (with removal of electrodes) results; correlation coefficients ranged from r=0.81-0.94, p<0.01. Furthermore, Burdette and Gale (1990) reported between-session (with removal of electrodes) reliability for the masseter between 0.56-0.65 (Pearson's correlation 'r' values) and for the Temporalis 0.33-0.48 (Pearson's correlation 'r' values). However, the use of the 'r' value as an indicator for reliability is potentially problematic, as this is a measure of the linear correlation of two variables; it is expected that the same measurement (i.e. masseter muscle activity) would correlate between two sessions, even with a considerable period of time between data collection. A less problematic statistical analysis would be an Intraclass Correlation Coefficients (ICC), which considers the values as re-tests of the same measurement and therefore indicates the strength of obtaining the

same outcome value from the same process. Suvinen et al. (2009) reported Intraclass Correlation Coefficients ranging from 0.87-0.89 for the masseter and anterior temporalis during clenching, between sessions (with removal of electrodes). Castroflorio et al. (2008) highlighted that a well-controlled protocol and procedure for electrode placement are fundamental for improving the reproducibility of results, which must be verified prior to use in large scale studies. The following section will describe the pilot study used to verify the suitability of the electrode placement on the jaw elevator muscles for use within this thesis. The reliability of the EMG protocol will be addressed in Chapter 2 Section 2.4 'Can Masticatory Electromyography be Normalised to Submaximal Bite Force?'

Electrode Placement Pilot Study: Aims and Objectives

The aim for the pilot study investigation was to assess the suitability of surface EMG electrode positioning for measuring jaw elevator muscle activity during a bite force study. The objectives were to;

(i) Conduct an EMG electrode placement case study to identify muscle activity when clenching and non-clenching facial expressions were performed.

(ii) Identify non-clenching facial expressions that either utilise the masseter and anterior temporalis muscles or cause crosstalk to register as muscle activity at the masseter and anterior temporalis.

(iii) Use the findings of the case study to inform the protocol used during the full investigation (Chapter 2 Section 2.6).

Process

Prior to commencement of the study ethical approval was obtained and granted from the Department of Exercise and Sport Science Research Ethics Committee, Manchester Metropolitan University. The participant gave informed written consent to take part in the study. Masseter and anterior temporalis EMG muscle activity during various clenching and non-clenching facial expressions were recorded on one female participant aged 22 years old, who was recruited on campus at Manchester Metropolitan University, Cheshire. The participant sat comfortably upright on an office style chair. Surface EMG sensors (manufactured by Delsys, Boston M.A., USA) were placed bilaterally over the main portion of the masseter. Specifically, 20mm from the inferior edge of the mandibular angle, in a straight line to the point where the external angular process of the frontal bone meets the frontal process of the zygomatic bone, a similar placement orientation to the study of Castroflorio et al. (2005). This line runs parallel to the superficial masseter muscle fibres. Surface EMG sensors were also placed bilaterally on the anterior portion of the temporalis, inferior to the junction of the external angular process with the frontal process of the zygomatic bone. The sensors were placed posterior and parallel to the eyebrow line, so that the electrode bars were perpendicular to the anterior temporalis muscle fibres (Figures 2.27 and 2.33-2.35). Prior to attaching the electrodes, the areas of

skin were shaved if necessary, and cleaned with an alcohol wipe. During clenching, the positioning of the electrodes was checked through palpation.

The participant was asked to perform eleven simple facial expression tasks. Prior to recording EMG activity, the facial expression movements were explained to the participant and were demonstrated by the investigator. They were also provided with a mirror to check their movements and adequate time to practice, prior to data collection. The following facial expressions were recorded:

- Forehead raise
- Frowning
- Blinking
- Open/Scrunch eyes
- Lateral mandibular shift left
- Lateral mandibular shift right
- Protrusion
- Rapid mouth open
- Slow mouth open
- Sustained clench on teeth
- Sustained clench on a buffer

The participant performed each movement (either sustained or repeated) for the duration of the 10s recording, which was visible to the participant on a computer monitor as a feedback tool. Each expression was performed only once, but sufficient time was provided between recordings for the participant to practice the next expression. There was no formal data analysis conducted on the preliminary EMG recordings; the trace from each EMG was used simply as to ascertain which facial expressions produced detectable activity.

Results

The facial expressions and movements performed produced varied EMG activity, which consisted of either discrete periods of activity or continuous low level activity. The results that follow (Figures 2.1-2.11) show the EMG trace from each facial movement with details of the active muscles.

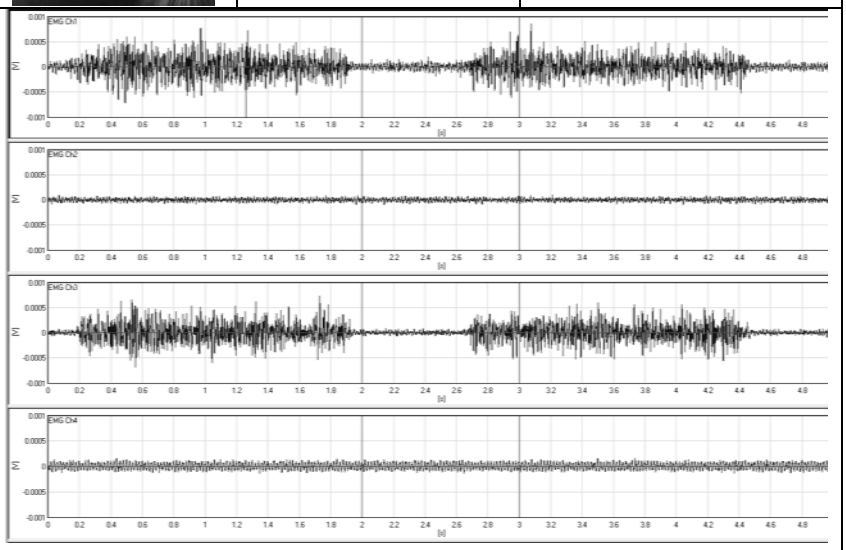
Expression	Example	Movement	Muscles recruited
Forehead raise		Inner corner of eyebrow raised Outer corner of eyebrow raised	Frontalis
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.1: Muscle activation of the Masseter and Anterior Temporalis during a forehead raise.

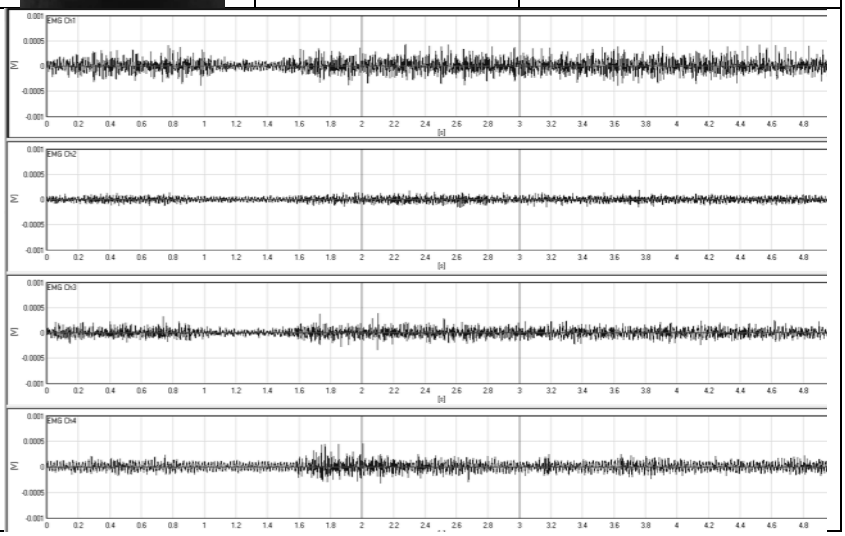
Expression	Example	Movement	Muscles recruited
Frowning		Eyebrows drawn medially and down	Corrugator supercilii, Depressor supercilii
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.2: Muscle activation of the Masseter and Anterior Temporalis during frowning.

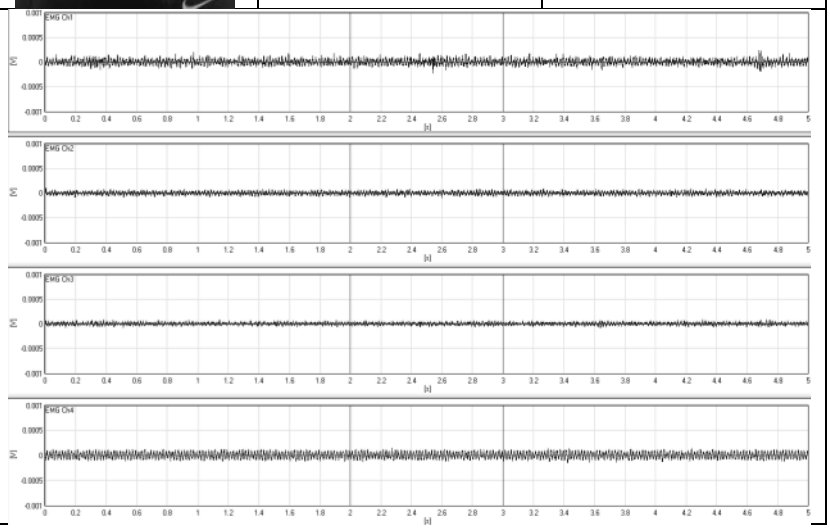
Expression	Example	Movement	Muscles recruited
Blinking		Natural blinking; gently closing and opening eyes simultaneously	Relaxation of levator palpebrae superioris; orbicularis oculi, pars palpebralis
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.3: Muscle activation of the Masseter and Anterior Temporalis during blinking.

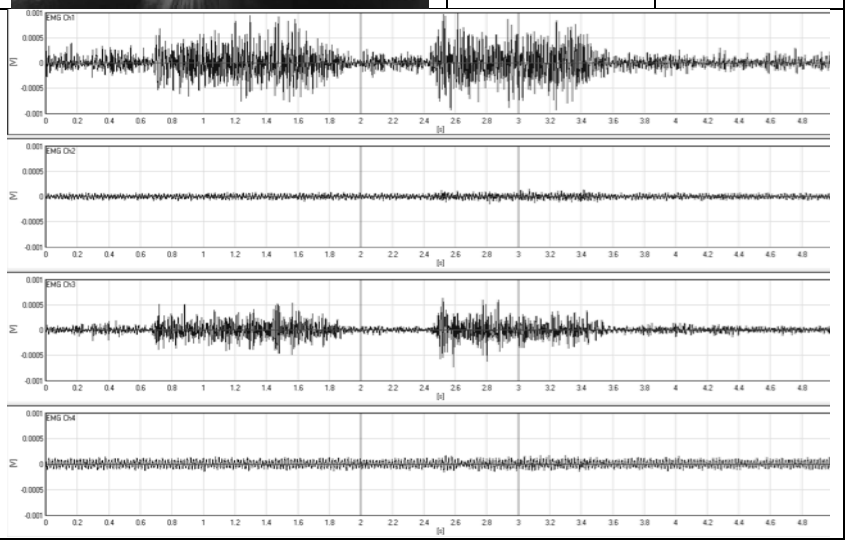
Expression	Example	Movement	Muscles recruited
Open/Scrunch Eyes		Eyes widened Cheeks raised; eyes narrowed	Levator palpebrae superioris Orbicularis oculi, pars orbitalis
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.4: Muscle activation of the Masseter and Anterior Temporalis during opening and scrunching eyes.

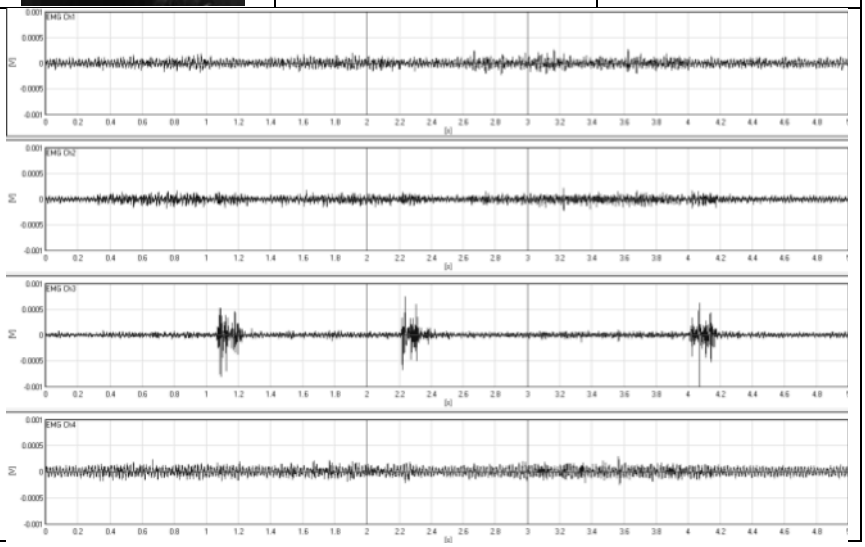
Expression	Example	Movement	Muscles recruited
Lateral Shift Left		Mandible moved to the left, out of alignment with maxilla.	Left Masseter, Temporalis and digastric. Right medial and lateral Pterygoid.
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.5: Muscle activation of the Masseter and Anterior Temporalis during a left mandibular lateral shift.

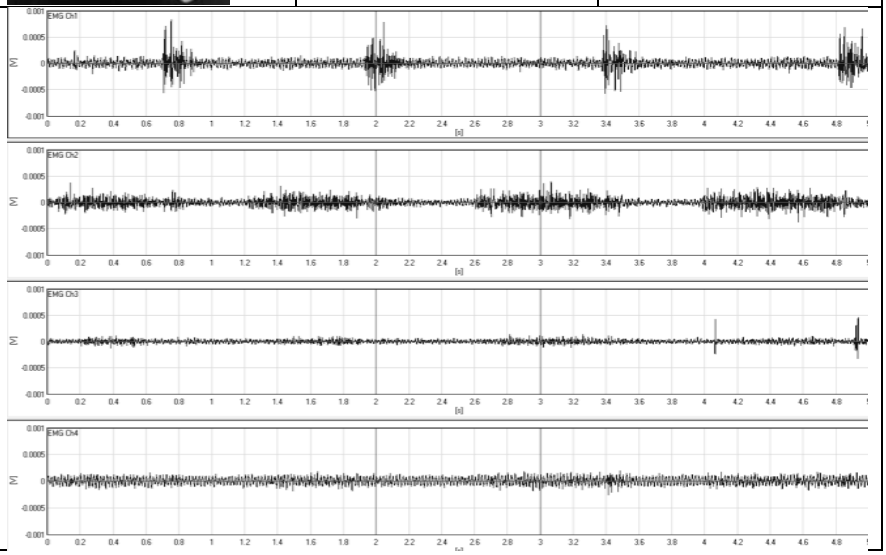
Expression	Example	Movement	Muscles recruited
Lateral Shift Right		Mandible moved to the right, out of alignment with maxilla.	Right Masseter, Temporalis and digastric. Left medial and lateral Pterygoid.
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.6: Muscle activation of the Masseter and Anterior Temporalis during a right mandibular lateral shift.

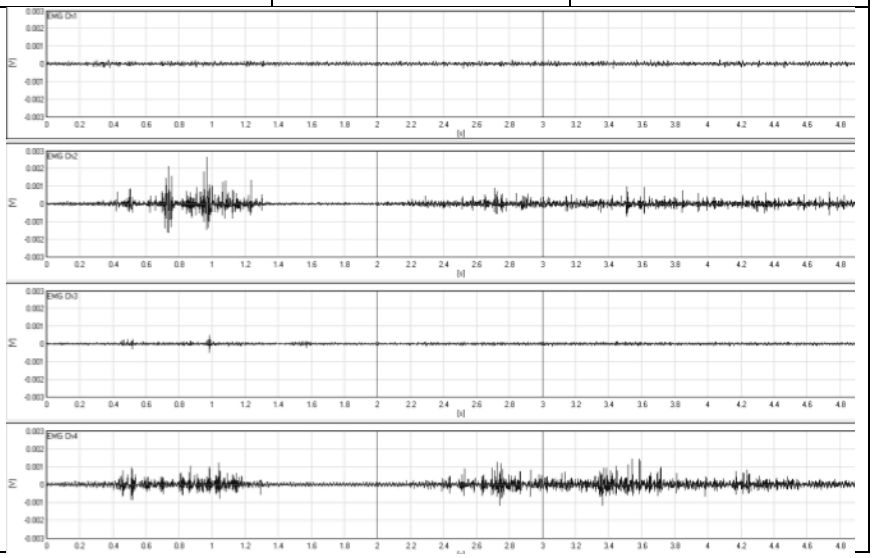
Expression	Example	Movement	Muscles recruited
Protrusion		Lower teeth and mandible project forward beyond the upper teeth and maxillae.	Pterygoids
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.7: Muscle activation of the Masseter and Anterior Temporalis during protrusion of the mandible.

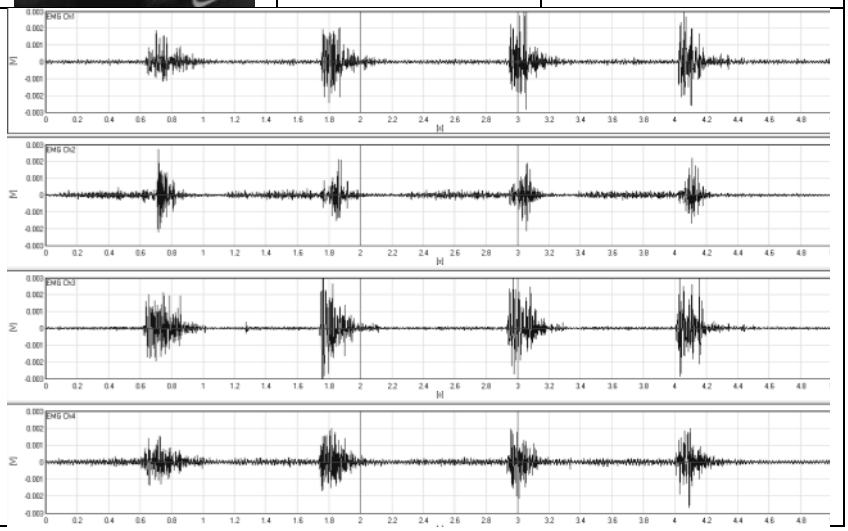
Expression	Example	Movement	Muscles recruited
Rapid open		Mandible dropped Mouth stretched open	Deep Masseter, posterior Temporalis, Medial and lateral Pterygoids, digastric, Geniohyoids and Mylohyoids.
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.8: Muscle activation of the Masseter and Anterior Temporalis during rapid jaw opening.

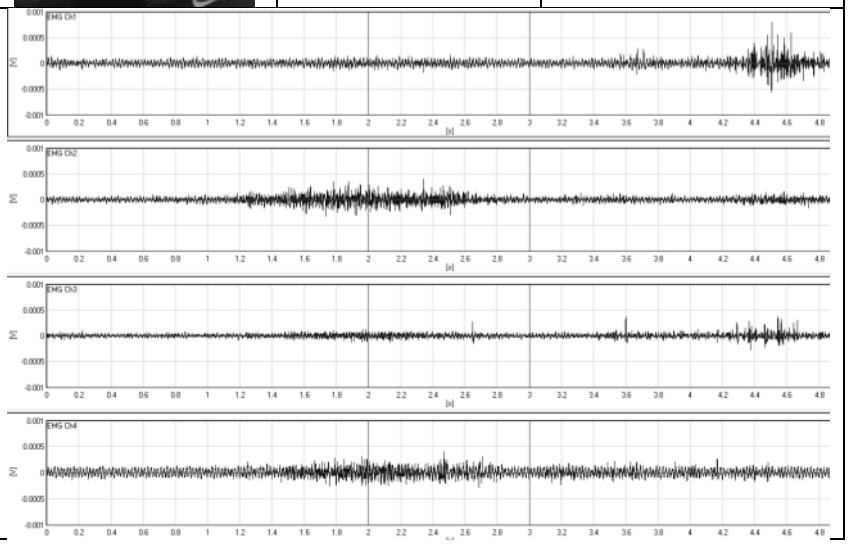
Expression	Example	Movement	Muscles recruited
Slow Open		Mandible (controlled movement) opened Mouth stretched open	Deep Masseter, posterior Temporalis, Medial and lateral Pterygoids, digastric, Geniohyoids and Mylohyoids.
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.9: Muscle activation of the Masseter and Anterior Temporalis during slow jaw opening.

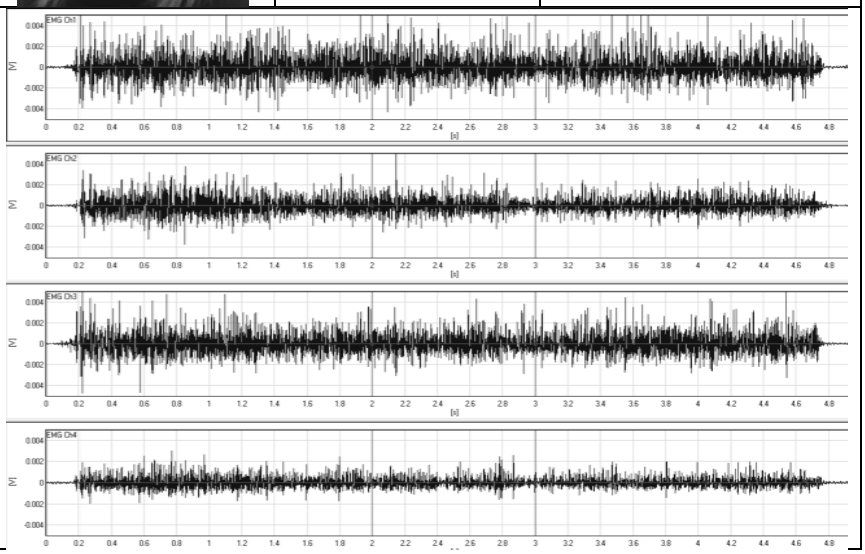
Expression	Example	Movement	Muscles recruited
Sub-maximal sustained Clench		Teeth pressed together in occlusion	Superficial and deep Masseter, Anterior and posterior Temporalis. Medial and lateral Pterygoids.
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.10: Muscle activation of the Masseter and Anterior Temporalis during a sub-maximal sustained clench.

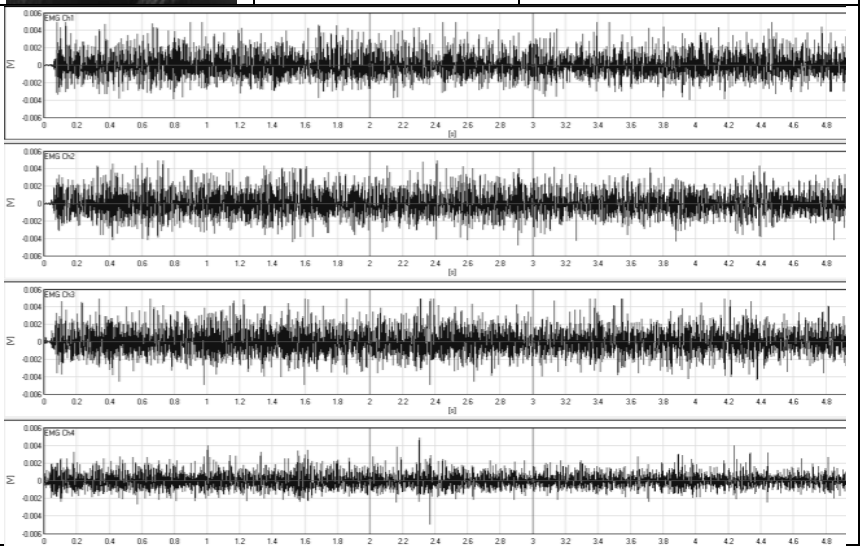
Expression	Example	Movement	Muscles recruited
Sub-maximal sustained clench with buffer		Posterior teeth pressed into buffer, anterior teeth open.	Superficial and deep Masseter, Anterior and posterior Temporalis. Medial and lateral Pterygoids.
EMG Trace Left Anterior Temporalis Left Masseter Right Anterior Temporalis Right Masseter			

Figure 2.11: Muscle activation of the Masseter and Anterior Temporalis during sub-maximal sustained clench onto a buffer.

Discussion

This investigation aimed to assess the suitability of the positioning of surface EMG electrodes for use in a muscle activity study. Specifically, the case study intended to explore whether activity was detected at the jaw elevator muscles during the performance of non-clenching facial expressions. The pilot study aimed to identify facial expressions that either utilise the masseter and anterior temporalis, or cause crosstalk to be registered as masseter and anterior temporalis muscle activity. The

vast majority (Figures 2.1, 2.2, 2.4-2.11), of movements, all except blinking (Figure 2.3), caused muscle activity to be registered, which indicates any additional facial expression performed during biting may give a false EMG. Siéssere et al. (2009) examined similar jaw movements to measure muscle activity of the masseter and temporalis; their study compared muscle activity during lateral divergence of the mandible with muscle activity during maximal bite forces. They found that EMG activity could be easily detectable at the temporalis and especially at the masseter during mandibular movements, even in the absence of deliberate clenching. These findings are in accordance with the results from the present pilot study.

The movements that involved clenching registered clear muscle activity across all four of the muscles, which indicates a suitable electrode placement for measuring muscle activity during clenching or biting. However, only two of the eleven expressions recorded during this study qualified as intercuspal clenching, but ten of the eleven expressions registered activity at either one or both of the jaw elevator muscle sensors. Therefore, a jaw elevator EMG study must incorporate specific instructions to participants to prevent additional facial expressions whilst clenching. Despite the superficial positioning of the elevator muscles, the probability of detecting crosstalk is high because the facial muscles overlap one another, both spatially and in terms of activity patterns (van Boxtel, 2010). Not only are the facial muscles preferentially activated according to task, they also possess different shortening velocities and periods of eccentric and concentric contraction (Koolstra and Van Eijden, 1997). The techniques and equipment used in the present study were not able to isolate specific muscles to quantify crosstalk, and as stated earlier,

the needs of the study did not warrant the use of invasive techniques. Regardless of the conflicting evidence for the amount of crosstalk that may contaminate sEMGs (De Luca and Merlettis, 1988; Solomonow et al., 1994), a stringent placement protocol and clear instructions for participants to remain expressionless whilst clenching, is essential. Additionally, the fixed electrode distance of 10mm is slightly larger than some facial studies which have used 8mm (Lapatki et al., 2003) but smaller than other studies that have successfully measured EMG at the masseter and temporalis using an inter-electrode distance of 30mm (Saifuddin et al., 2001; Suvinen et al., 2009). It is pertinent to suggest that because previous research has successfully used electrode of similar sizing, the Delsys EMG system (Delsys, Boston M.A., USA) electrodes are suitable for facial EMG recording. In addition, the muscle activity data can be accurately integrated in time-sync with the bite force data, which is an added benefit of using the present system. The pilot study aimed to identify a suitable protocol for measuring jaw elevator muscle activity and to reduce crosstalk from neighbouring muscles. The findings of the case study recommend that future investigations of jaw elevator muscle activity (especially during biting) will use sEMG and specific instructions to the participant to relax any facial expressions whilst biting, during both maximal and submaximal tasks. This is concurrent with previous research that ensured participants remain relaxed throughout the experimental protocol (Tingey et al., 2001).

2.3 Justification and Selection of the Bite Force Measurement Technique.

Introduction

There is a plethora of commercial force measuring devices available that have been used in previous research studies, combined with the numerous approaches to construct novel devices that have been reported in the literature, which provide a vast array of choice for researchers and engineers alike (see Table 2.1). There is substantial opportunity to conduct investigative research based on pre-existing ideas and commercially available products. However, where choice and opportunity are rife, comparison and evaluation between such diverse products and techniques are rarely simple or conclusive, even though the outcome of both techniques are force measurements. Moreover, force-measuring devices are usually required to undergo a calibration process, mechanical testing and/or connect to a digital handling tool to convert or display the key variables (Flórez and Velásquez, 2010). The range of calibration processes used by authors, in part reflects the variety of devices available (differing size, shape, surface area), and is in part due to the range of specifications provided by manufacturers on the loading limits of each device (Bensen and An, 2000). As such, there is no set approach used, or is necessarily suitable for every device measured.

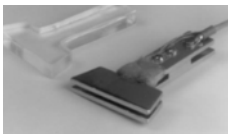
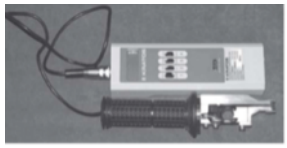
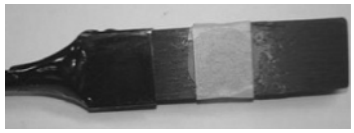
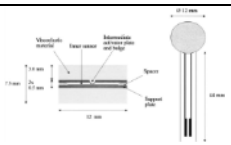
Author	Device	Image †
Tortopidis et al. (1999)	Strain gauge technology 2 stainless steel beams Self curing epoxy resin (shape of dental contacts)	
Palinkas et al. (2010)	Digital dynamometer	◆ 
Trawitski et al. (2011)	Digital gnathodynamometer Two rods with teflon disks for biting on	
RoldÁn er al. (2009)	Dual beam uni directional transducer Carbon fibre in epoxy resin 4 strain gauge sensors	
Fernandes et al. (2003)	Force Sensing Resistors Added stainless steel activator plate and 'bulge' to convert to N	
Shinogaya et al. (2001)	Dental pre-scale Non electronic – post bite scanning	◇ 
Throckmorton et al. (2009)	Records distribution of relative forces around the tooth row Not absolute forces	

Table 2.1: A selection of bite force devices reported in literature.

† All figures were obtained from the corresponding reference, with the exception of ◆ (Calderon et al., 2006) and ◇ (Alkan et al., 2008).

Calibration of bite force devices

Bite force study devices fall generally into four categories: (i) transducer or strain gauge devices (ii) dynamometers (iii) dental pre-scale and (iv) force sensing resistors (FSR's). Each individual device is configured differently depending on the manufacturer's specifications, but the technical theory on which they are founded is common to many products, commercial and novel.

Transducers used for bite force measurements are commonly constructed from two stainless steel arms, fitted with foil strain gauges (Fogle and Glaros, 1995; Kiliaridis et al., 1995; Tortopidis et al., 1998; Ferrario et al., 2004; RoldÁn et al., 2009). Tortopidis et al. (1998) reported that they calibrated their device using standard weights prior to collection of experimental data. Their study reported a non-significant difference between sessions, therefore the device was deemed linear and consistent between sessions, but no further details concerning the calibration process were reported. Ferrario et al. (2004) used a stainless steel strain gauge transducer which also reportedly presented a low (±2%) error between 50-350N (at room temperature), however the method of calibration was not reported. Similarly, a study conducted by Fogle and Glaros (1995) used a specially constructed metal fork device, instrumented with strain gauges but they did not state any information regarding the calibration processes. Furthermore, Kiliaridis et al. (1995) developed an interocclusal metal fork-like device incorporating strain gauges, which connected to a speedomax recorder (paper speed 20cm/min), with a maximum pressure capacity of 980N. Similar to Tortopidis et al. (1999), the fork-like device was calibrated using known loads but a

detailed explanation of the process was not reported. Kiliaridis et al. (1995) does further report that the bite force device was calibrated regularly to obtain a linear relationship between load and recorded deflection. RoldÁn et al. (2009) similarly measured maximum bite force using a specially designed dual-beam, unidirectional transducer. However, unlike previous studies, RoldÁn et al. (2009) constructed the device from carbon fibre in epoxy resin with four strain gauge sensors placed to optimally record bite force; neither the calibration process nor the calibration results were detailed in the paper. Raadsheer et al. (2004) used a three component force transducer capable of registering both the magnitude of the bite force and the plane(s) in which the force was generated, this device was previously used in studies by van Eijden et al. (1988) and van Eijden (1990). The study used calibration data provided by the manufacturer, however following modifications, van Eijden et al. (1988) placed know weights (10-200N) on the device in different directions. From this, there appeared to be no alteration to the performance of the product, indicating a stable and consistent calibration. Moreover, studies that used a U-shaped force transducer with rosette strain gauges, developed by Paphangkorakit and Osborn (1997) and a miniature load cell transducer with digital display meter, developed by Burnett et al. (2000) did not report within their study any form of calibration procedure. Pereira et al. (2007) used a pressurised tube as a bite force device, which measured changes in pressure. They simply reported the accuracy (0.1N) of converting pressure in psi to N from the pressurised tube transducer. Müller et al. (2001) reported the linearity (1-1000N ±1N) of their custom-made load cell with full bridge strain gauge with no mention of the process used. Nevertheless,

their study compared bite forces in denture wearers with lower ridge resorption who were capable of bite forces no greater than 180N. Furthermore, Mioche et al. (1993) used two miniature load cells, one with a range of 100N (±50% load range), the other 250N (±50% load range). Both load cells reportedly had a sensitivity corresponding to 1% and a function that allowed the load cell to be set to zero, however, there was no detailed procedure for calibration prior to the study. It would appear from the literature, that only a small percentage of studies report their calibration process. Some studies conducted using common fork-like transducer devices or unusual experimental devices, provide little or no information concerning calibration procedures prior to human use. The reasons for this are unknown, but as the sources of variation differ with each experiment (RoldÁn et al., 2009) it is important to understand the basic load/output relationship of each device, if comparisons between the findings of the studies are to be drawn.

Within dynamometer based bite force studies, Palinkas et al. (2010) details a 1000N capacity device which operates using two parallel arms and a high precision charge cell; the apparatus has a set-to-zero key and a digital display. Although the configuration of the device is similar to that of the common transducer, there is little explanation of the scientific principles underpinning the recordings. The use of this device has been reiterated in Trawitzki et al. (2011) and Siéssere et al. (2009), but as with Palinkas et al. (2010), there was no further mention of calibration procedure. Furthermore, bite force studies that use dental pre-scale or pressure sensitive film mention very little if any to the degree of error, mechanical calibration or calculation (Sato et al., 1999; Sondang et al., 2003), except Shinogaya et al. (2001) who found

the method error to be 5.3% for maximal clenching force. Pressure sensitive film can only be used once before discarding, as it is designed to deform under load and the level of deformation indicates the amount of pressure applied to the film. For example, Brimacombe et al. (2009) compared the accuracy of a calibration process recommended by Teckscan to the accuracy of two user-defined calibration processes. The study recommended user-defined calibration methods and calibration of sensors individually to improve accuracy of results. Throckmorton et al. (2009) validated numerous hand held T-scan devices using bespoke loading apparatus with fixed dentures; incremental loads were applied through the dentures to the T-scan device, whilst protective coverings were experimented with in order to convert force distribution into absolute force values. Similarly, Brimacombe et al. (2009) calibrated Tekscan pressure sensors using a universal testing machine and the Tekscan calibration software. They also compared the accuracy of the manufacturers calibration system with their own, more detailed mathematical calibration curves; the accuracy of their curves were reportedly almost 5 times more accurate (RMS error of 0.6%) than those provided by the manufacturer (RMS error of 2.7%). These findings indicate the importance of conducting a calibration process on bite force equipment, regardless of manufacturing specifications, to ensure the data is as accurate as possible prior to the data collection procedure.

In general, studies conducted on Force Sensing Resistors have included details regarding the calibration and mechanical testing. Flórez and Velásquez (2010) included detailed graphs on static creep, dynamic hysteresis and moving integral algorithms used to correct for each. Furthermore, Hollinger and Wanderley (2006),

conducted an evaluation of force-sensing resistors; they reported the method and results of three types of static weight test and a ramped force (hysteresis) test across three different commercially available FSRs. The study found differences in precision, linearity and time responses between the devices, indicating a need for specific calibration prior to use. Force sensing resistors have a non-linear relationship with applied force; due to the nature of their configuration they display an exponential output with increasing force (Zehr et al., 1995), which may compromise the accuracy of each measurement. Despite this inherent non-linear nature, Zehr et al. (1995) calibrated FSRs by loading and unloading various loads through a piston rod, which pressed down uniformly on the surface of the FSR. Subsequently, the data was corrected with a linear least means squares equation to account for creep however, evidence of hysteresis remained even after corrections. The voltage during loading was consistently higher than during unloading. Moreover, Hall et al. (2008) reported that the application of a 4^{th} order polynomial equation, considerably improved the output of compressive forces in comparison to the applied force. They concluded that their method was adequate for eliminating hysteresis. Regardless of accuracy, the method used by Hall et al. (2008), is unsuitable for studies that require immediate, real-time feedback, because the mathematical alterations are applied after the data is recorded, therefore the participant cannot view their bite force in real time. Conversely, Fernandes et al. (2003) developed a novel sensor specifically for bite force measurements; they applied a steel activator plate depression bulge to an FSR to specify the contact area on the surface of the FSR and thereby ensure that the input was accurately converted from pressure to force. The device was wrapped

in a silicone dental impression material to make it suitable for use in the mouth. Fernandes et al. (2003) calibrated the device against a universal testing machine; compressive loading was conducted within the ranges of 10-500N either in a stepwise format or continuously. Furthermore, the unloading portion was recorded for calculation of hysteresis and linearity. They then applied a least squares 5th order polynomial regression equation which resulted in accurate data measurement from 20 to 550N. From the present literature, it is apparent that the calibration of FSRs using static loads will exhibit hysteresis and the addition of mathematical equations to FSR voltage output is necessary, in order to compensate for hysteresis and to calculate exact force (Zher et al., 1995; Fernandes et al., 2003; Hall et al., 2008). Devices used in bite force studies offer an array of benefits; including low cost force sensing resistors (Zehr et al., 1995), unobtrusive pressure sensitive film (Sondang et al., 2003) and conveniently, commercially constructed digital dynamometers (Siéssere et al., 2009). However, due to the large number of possible devices, the reliability of intra-oral bite force measurements remains questionable because the findings are often difficult to compare due to variation within the technique (RoldÁn et al., 2009).

Aims and Objectives

The present study will examine the suitability of both force sensing resistors (FSRs) and button style compression load cells (LCs) for measuring force during loading. Furthermore, the equipment that is most suitable for measuring force for use in an

immediate feedback system to the participant will be developed and calibrated for use in the main investigation of this thesis. The objectives are (i) to calibrate two force sensing resistors (FSRs) and two button style load cells (LCs) to known loads, by the use of a mechanical testing machine (ii) to identify which device can produce a linear response to increased load and (iii) to evaluate the results of both calibration procedures to determine the most suitable device, in terms of repeatability/reproducibility and visual feedback for the participant.

Materials and Method

Force Sensing Resistors

Two 18.3mm diameter force sensing resistors (RS Components Ltd., Northamptonshire, UK) were each soldered to a foot switch connection lead and pre-amplifier (Delsys, Boston M.A., USA). The Delsys foot switch connection lead is designed to attach to a Delsys FSR membrane; however, a more suitably sized FSR membrane was purchased for the purpose of this study, which connected in its place. The overall dimensions of each FSR sensor were width= 6.2mm length= 54.14mm diameter(ø)= 18.3mm x 1mm thick. The manufacturers specification reported an optimum pressure range of 0.07 to 7 bar; a resistance of 10MΩ to 1 kΩ; a maximum applied pressure of 35 bar; signal return time of 1-2 ms; an operating temperature of -30°C to +170°C; and a measurement repeatability of ±2%. As shown in Figure 2.12, the FSRs were each labelled and calibrated separately to ensure accurate translation of the results.

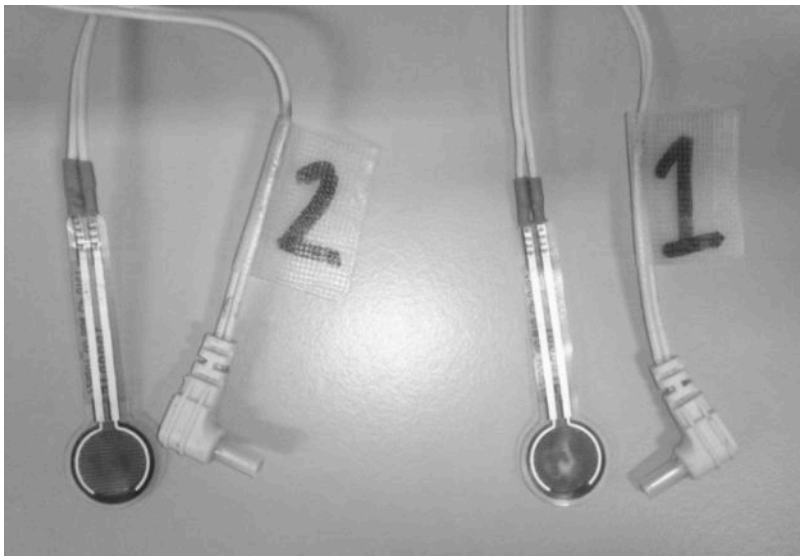


Figure 2.12: Force Sensing Resistors individually soldered to a Delsys foot switch.

Button Style Compression Strain Gauge Load Cells

Two button style compression load cells (Omega Engineering Ltd., Manchester, UK), which operate using strain gauge technology, were each connected to a Delsys pre-amp lead via a bespoke amplifier (Figure 2.13). The amplifier served to amplify and convert the LC signal into a voltage output that was compatible with Delsys software. Dimensions of each LC were 3.8mm H x 13mm(ø), and the inner button measuring 0.51mm H x 3mm(ø), each were labelled and calibrated separately as shown in Figure 2.14.

Figure 2.13: Custom-made amplifier that connects the load cell to the Delsys Bagnoli system.

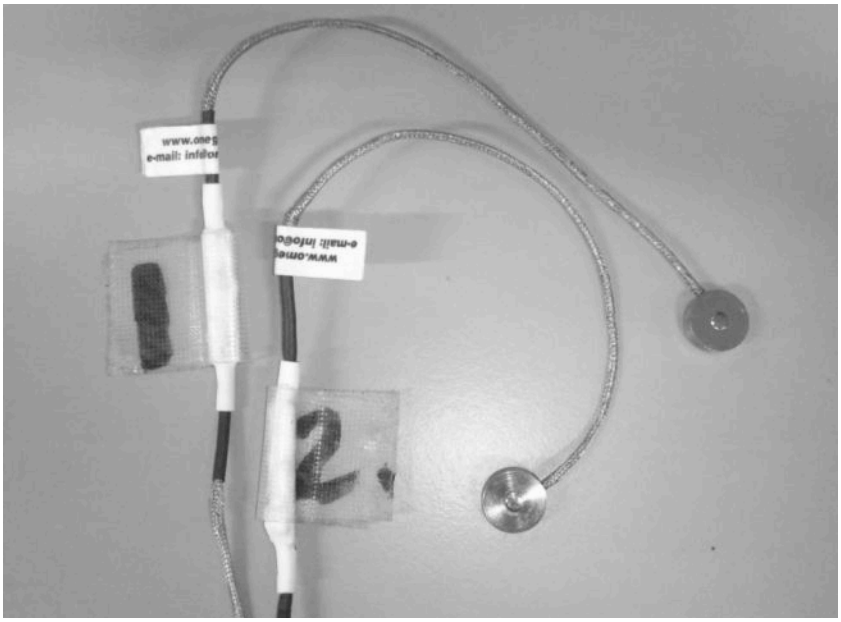


Figure 2.14: Button-style compression load cells, without protective covering.

Figure 2.15: Lloyd Universal testing machine with 5kN load cell and custom made cylindrical loading arm with FSR in place.

The FSR's and LC's were connected to the Delsys EMG system for the purpose of viewing and recording the voltage signal in real time, additionally the system was intended to allow for total synchronization of both force and muscle activity recordings. Once connected, the devices were individually calibrated using a series of known incremental loads (0-800N) by means of a LRX plus Materials Testing Machine (Lloyd Instruments Ltd., Hampshire, UK) fitted with a 5KN load cell and a custom made cylindrical loading arm of 18mm (Figure 2.15). Specific static loads (described in the next section) were applied to both sensors in turn whilst the subsequent voltage was recorded using a Bagnoli 8-Channel EMG system (Delsys,

Boston M.A., USA) then stored and analysed on a HP laptop (Figure 2.16). Both the FSR's and LC's were calibrated independently, with no additional material covering or attachments. They were then tested with the addition of Ethyl Vinyl Acetate (EVA) material (Bracon Dental Supplies, East Sussex, UK). EVA is a common polymer, which is used to fabricate mouth guards and dental splints. Each sensor was encapsulated between two sections of translucent EVA; the dimensions of each piece measured 25mm W x 35mm L x 1.5mm thick (making a total FSR thickness of approx. 4mm) (Figure 2.17). The load cells followed the same procedure as the FSRs, however each were additionally fitted with a stainless steel disk (2mm H x 13mm (ø)) sandwiched between the load cell (button) and the section of EVA. This was to create greater surface area contact of the compression button, and resulted in a total LC thickness of 8.8mm (Figure 2.18).

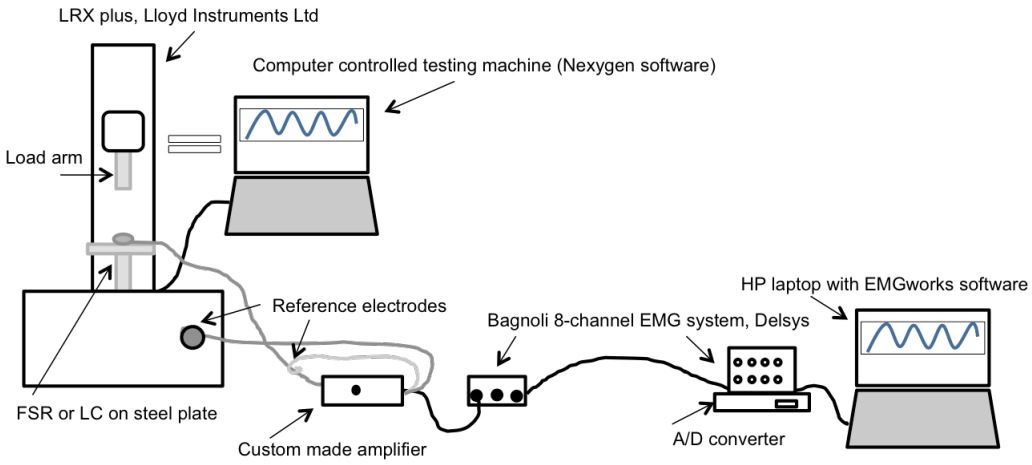


Figure 2.16: Schematic diagram of the calibration set-up for the bite force sensor.

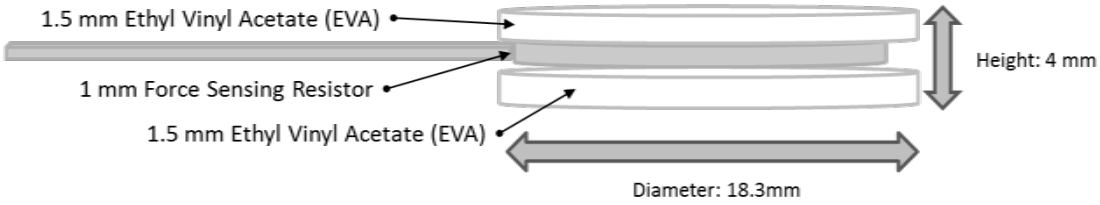


Figure 2.17: Schematic diagram of FSR arrangement with EVA

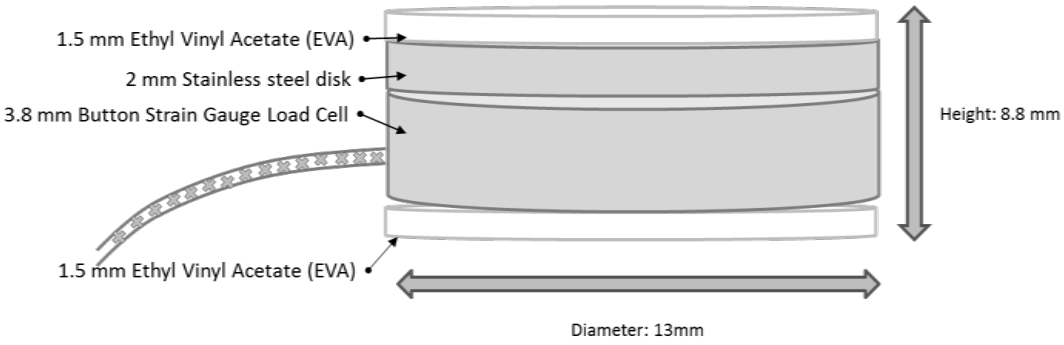


Figure 2.18: Schematic diagram of LC arrangement with EVA

Data Collection

During all test conditions, the force sensor or load cell was fixed in place on the flat stainless steel plate of the Lloyd testing machine, directly below the 18mm diameter cylindrical loading arm (Figure 2.16). Each device was fixed securely with electrical insulation tape to prevent it from moving during the compression loading calibration process. The circumference of the load arm was aligned with the circumference of the sensor so that the force was applied directly through the sensor.

Both the FSR's and LC's were calibrated from 0-800N at 100N increments in static loading both individually (bare) and with the additional EVA coverings. The maximum load was based on the information provided in the product specifications and the equipment configuration; both devices had a 1kN capacity, which is suitable for human bite forces that are commonly between 200 and 700N throughout adult life (Bakke et al., 1990). However, both were calibrated to the lower force of 800N to avoid potential damage from over loading. For each device at every 100N increments, the materials testing machine was set to increase from 0 to the intended set peak load steadily over the duration of 1 min, held for 20s, then loading was released. During peak force hold, the voltage signal from the device was captured on the Delsys EMG works Acquisition software. This testing process was conducted on two separate days to assess the repeatability of the sensors.

Software Calibration

After the initial incremental calibration tests were conducted, an updated version of the Delsys software became available, which allowed the LC's to be calibrated into the Delsys software, using the same static protocol. However, each LC was calibrated during 50N increments from 0-800N, this was done to provide a greater level of accuracy from the Delsys software. The software programme was set to record and save the voltage readings of both LCs (on separate channels) for each user-specified load (N) in order to convert each voltage signal directly to newtons. Once complete, this resulted in the Delsys Acquisition screen displaying newtons rather than Volts on

the Y-axis in real time. Although this calibration process was used during the main investigation, the data presented and discussed in this section is the original calibration study data.

Data Analysis

The FSR and LC data was collected and recorded as Volts using EMG works 4.0 Acquisition, then transferred to the EMG works 4.0 Analysis software (Delsys, Boston M.A., USA) in order to export the data for further analysis. Loading data was recorded in real time using the NEXYGEN software (Ametek, P.A., USA). Both the voltage data sets from Delsys and the loading data sets from NEXYGEN were exported to Microsoft Excel (2010) for further analysis. A single voltage value that corresponded to the steady static load was identified for each 100N increment of static loading, for all measuring device conditions. The static incremental values were then exported to IBM SPSS Statistics 19 software for analysis. Statistical results were generated from a one-way ANOVA, where the dependent variables (measuring device with or without EVA) were compared across testing days. Furthermore, Intraclass Correlation Coefficients were used to assess the reliability of the re-test values for each measuring device with and without EVA. Levene's test for homogeneity (across all conditions) indicated equal variances ($p>0.05$) for all independent comparisons, therefore the assumption of homogeneity was met. Skewness and Kurtosis analysis indicated that the z-values for all variables were within the ±1.96 range, therefore they did not differ significantly from normality.

Results

The one-way ANOVA showed a mixture of significant and non-significant results for the FSRs and non-significant results for the LCs. Moreover, the F ratios reported by the ANOVA's were all positive (Figure 2.19-2.26), which indicates that the variance due to the experimental manipulations were larger than the variations due to random factors. Therefore it can be concluded, that the observed results were unlikely to have arisen by chance. The LCs showed no significant ($p>0.05$) between-session differences, but FSR1 did show a significant ($p<0.05$) effect of testing session. Furthermore, the ICC results show high between session reliability for both devices, but there is particularly less variance in the LC data (Table 2.2).

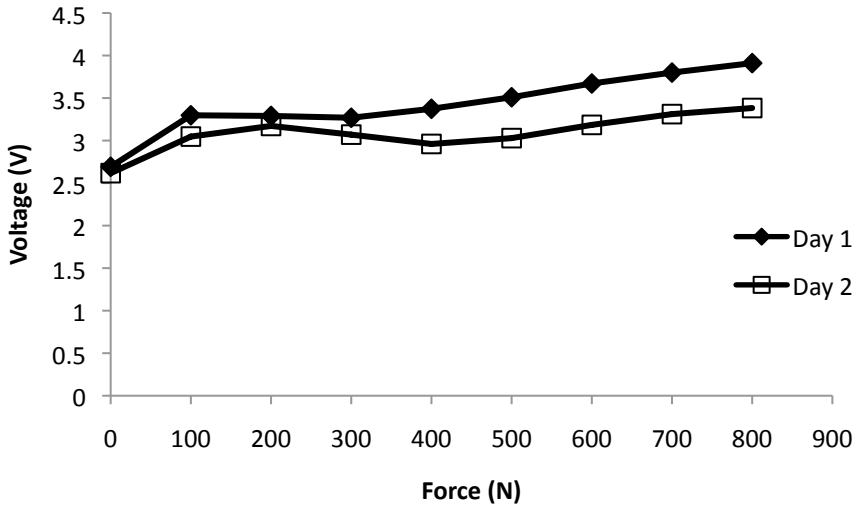


Figure 2.19: FSR1 (without EVA) voltage output plotted against force.

There was a significant effect of 'Day of Testing' on the Voltage output per load, $F(1, 16) = 5.696$, $p<0.05$.

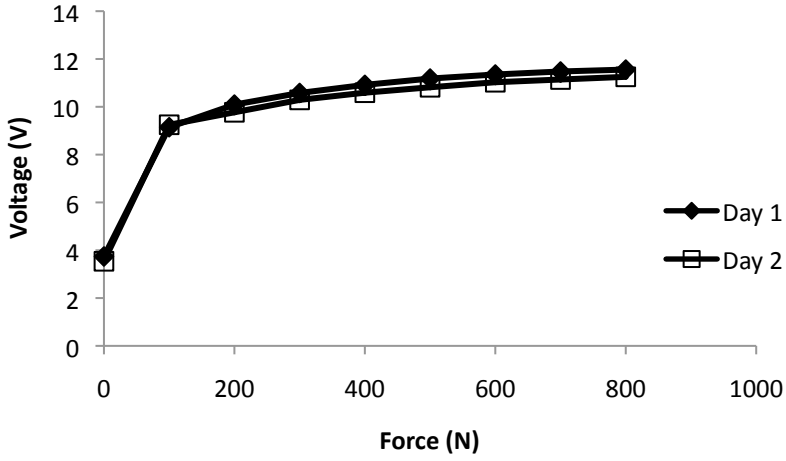


Figure 2.20: FSR1 (with EVA) voltage output plotted against force.

There was no significant effect of 'Day of Testing' on the Voltage output per load, $F(1, 16) = 0.052$, $p>0.05$.

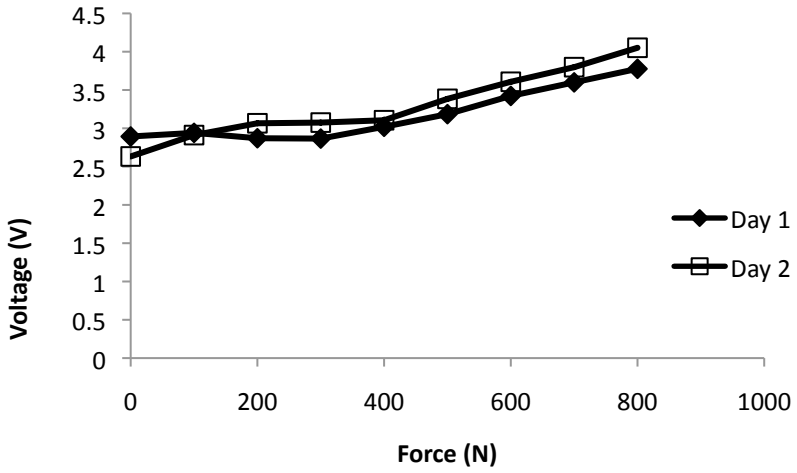


Figure 2.21: FSR2 (without EVA) voltage output plotted against force.

There was no significant effect of 'Day of Testing' on the Voltage output per load, $F(1, 16) = 0.382$, $p>0.05$.

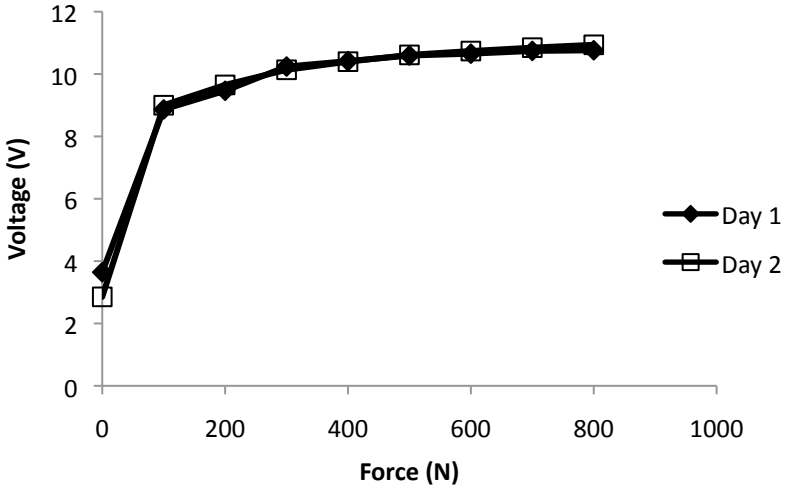


Figure 2.22: FSR2 (with EVA) voltage output plotted against force.

There was no significant effect of 'Day of Testing' on the Voltage output per load, $F(1, 16) = .000$, $p>0.05$.

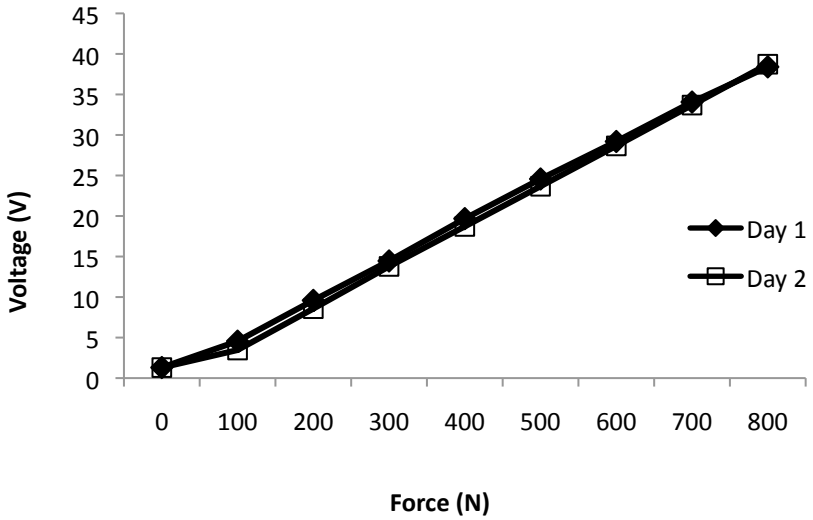


Figure 2.23: LC1 (without EVA) voltage output plotted against force.

There was no significant effect of 'Day of Testing' on the Voltage output per load, $F(1, 16) = .009$, $p>0.05$.

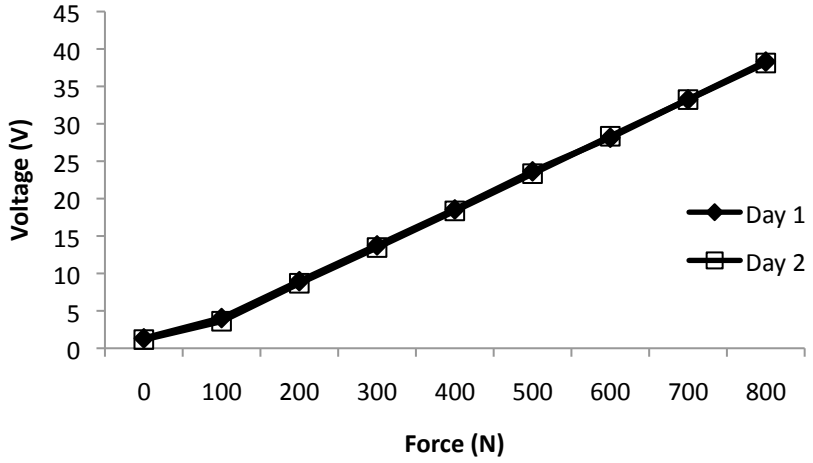


Figure 2.24: LC1 (with EVA) voltage output plotted against force.

There was no significant effect of 'Day of Testing' on the Voltage output per load, $F(1, 16) = .001$, $p>0.05$.

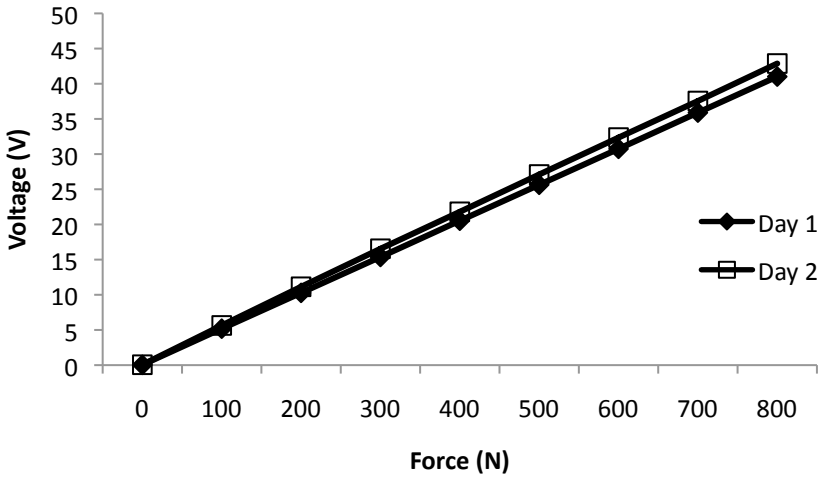


Figure 2.25: LC2 (without EVA) voltage output plotted against force.

There was no significant effect of 'Day of Testing' on the Voltage output per load, $F(1, 16) = .029$, $p>0.05$.

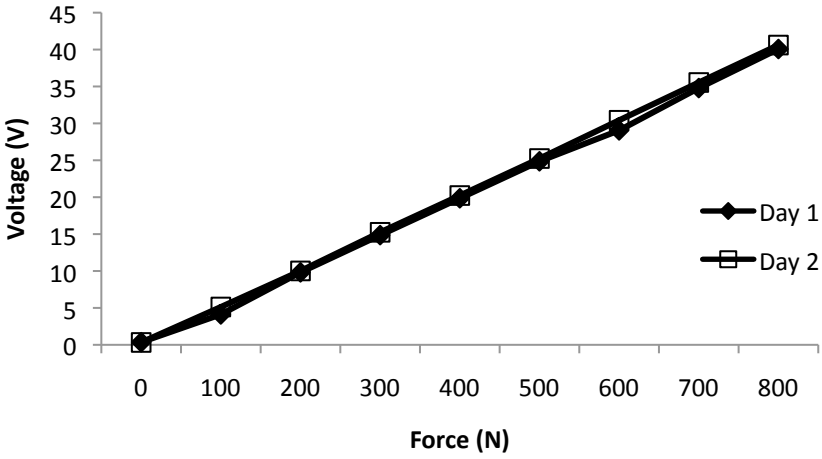


Figure 2.26: LC2 (with EVA) voltage output plotted against force.

There was no significant effect of 'Day of Testing' on the Voltage output per load, $F(1, 16) = .007$, $p>0.05$.

Intraclass Correlation Coefficients				
FSRs/LCs	ICC	95% CI Lower Bound	95% CI Upper Bound	Sig.
FSR 1 Bare	.677	-.212	.934	.002
FSR 2 Bare	.939	.677	.987	.000
FSR 1 +EVA	.996	.746	.999	.000
FSR 2 +EVA	.996	.985	.999	.000
LC 1 Bare	.999	.983	1.00	.000
LC 2 Bare	.998	.827	1.00	.000
LC 1 +EVA	1.00	.999	1.00	.000
LC 2 +EVA	.999	.984	1.00	.000

Table 2.2: ICCs for between session (Day1-Day2) calibration of the FSR and LC devices.

The ICC results shown in Table 2.2 indicate high (.677-.996) levels of reliability for the FSRs and high (.998-1.00) levels of reliability for the LCs. The 95% confidence intervals show a smaller amount of variation in the between session data in the LCs (.827-1.00) compared to the FSRs (-.212-.99).

Discussion

This study aimed to explore the linearity and reliability of force sensing resistors (RS Components Ltd., Northamptonshire, UK) and button style compression load cells (Omega Engineering Ltd., Manchester, UK) for measuring loading, with the view to developing a novel and reliable bite force device. The FSR's were easily connected to

the Delsys EMG system via a footswitch lead (Delsys, Boston M.A., USA), and were small enough to comfortably fit into the mouth without hindering bite force measurements even when covered with protective layers (4mm thick). The FSRs were inexpensive to purchase. Yaniger (1991) acknowledged the latter as a main benefit of the use of FSR's in research, whilst their accessibility and unobtrusive size and shape are acknowledged as beneficial in dental research for bite force measurements (Fernandes et al., 2003).

The present study found the FSRs produced different force/voltage relationship patterns when comparing bare equipment to that covered with EVA; the bare FSRs displayed a positive linear relationship that increased by ~1.5V over a range of 0-800N, whereas the FSRs with EVA showed an exponential relationship that increased by ~8V over a range of 0-800N. Buis and Convery (1997) stated that because FSRs are manufactured from a thin, compliant polymer, they may be affected by their supporting material, thus this may reflect the findings of the present study. Although the exact reason for the stark difference in present findings is not known, it is reasonable to suggest that the contact area between the thin surface of the FSR and the (microscopically coarse) steel loading arm was in fact reduced, in comparison to the compliant surface of the EVA. Similarly, Throckmorton et al. (2009) acknowledged this problem when measuring bite force using a bare sensor, the cusps of the teeth created very small areas of pressure across the sensor, rather than connecting the sensor with the whole tooth surface, thereby altering the force/voltage relationship. It is therefore important to calibrate FSRs as they would be used to measure bite force in the mouth, rather than in their bare, manufactured

state. The results from the present study show that FSRs covered with EVA produce an exponential force/voltage relationship, which is concurrent with literature and occurs because the polymer is not infinitely compressible, so the electrical conductance will saturate causing the resultant voltage values to become exponential (Zehr et al., 1995).

The between-day ANOVA statistical analysis showed no significant difference for both FSRs when covered in EVA and the ICCs were high (.996), which demonstrates their reliability between sessions. Fernandes et al. (2003) used FSRs to develop a novel bite force device, which was found to be 93% accurate when measuring repeatability. Conversely, Buis and Convery (1997) found FSRs displayed poor repeatability when measured without additional covering. The present study produced mixed findings regarding bare FSRs; FSR1 showed a significant difference between testing days without EVA ($p<0.05$), whilst FSR2 returned no significant difference ($p>0.05$) (Figures 2.19 and 2.21). Hall et al. (2008) found that bare FSRs had poor repeatability because the devices changed their resistance to loading through prolonged use. The mixed findings in the present study were unexpected, but it may be possible that FSR1 demonstrated significantly different results because it had been mechanically altered due to plastic deformation, more so than FSR2. This could be a hysteresis effect through prolonged use, as reported in Hall et al. (2008). However, the FSRs were intended to be used with the addition of EVA materials for measuring bite force. It remains imperative that FSRs are calibrated with additional covering, as they would be used in a full study, to improve accuracy and repeatability of the measurements (Throckmorton et al., 2009). The results (Figures 2.20 and 2.22)

showed that there was good repeatability between testing sessions with the addition of EVA, but the potential plastic deformation of the FSR remains problematic. Despite demonstrating repeatability with EVA covering, the FSRs still required corrections to provide immediate, accurate feedback to participants during bite force and to reduce the effect of hysteresis. Due to the mechanical composition of force sensing resistors, the intrinsically non-linear response to applied force requires additional corrections to be made to the output voltage values, to generate accurate absolute force values (Fernandes et al., 2003). This is also true of other studies, where the application of mathematical equations, in particular polynomial regression equations, of varying orders, has been essential to the production of meaningful results (Hollinger and Wanderley, 2006; Hall et al, 2008; Flórez and Velásquez, 2010).

The size of the compression load cells when covered with EVA was considerably larger than that of the FSRs; their overall height (8.8mm) resulted in an anterior incisal opening of ~12mm. This is considerably larger than the FSRs but is acceptably small, in accordance with another study (Paphangkorakit and Osborn, 1997) that found maximum bite force gradually reduced with incremental incisal opening heights from 9-32mm. Furthermore, Rues et al. (2008) used strain gauge bite force devices positioned over the molars and pre-molars; they reported a range of incisal opening height of 7.5-10.5mm during bite force . A review of bite force and occlusion conducted by Bakke (2006) suggests 15-20mm incisal opening height is optimum for maximal voluntary contraction of the jaw elevator muscles. However, Lindauer et al. (1993) reported a drop in muscle activity when molar separation was 9-11mm high.

Therefore, the present device was possibly not the optimum height for measuring molar bite force, but was within the appropriate range to measure bite force without dramatically altering the recruitment pattern of the jaw muscles. The load cells used in this study produced a linear force/voltage calibration relationship; this linear response to incrementally applied loading remained the same when covered with EVA. These findings imply that the protective covering had no effect on the devices response to loading, unlike the clear difference exhibited in the FSRs between bare and covered calibration. Tortopidis et al. (1998) found their transducer-based device to be linear, reliable and consistent between sessions, as did Kiliaridis, et al. (1995) who calibrated a strain gauge bite fork regularly to obtain a linear relationship between load and recorded deflection. Both studies are in accordance with the present study findings, the reported force ranges include 269-1039N (Tortopidis et al., 1998) and 565-728N (Kiliaridis et al., 1995) of healthy participants at baseline. The reported maximum bite force values detailed in 'Appendix D', also fit comfortably into the force range stated in the manufacturers guidelines for the LCs. The inter-session statistical analysis showed no significant differences between testing days for either LC1 or LC2, with or without EVA and the ICCs were exceptionally high (.998-1.00). Although the results are positive in comparison to that of the FSRs, there is little indication of error levels or statistical measures in the literature by which to compare the results of this study to other strain gauge based investigations.

To conclude, FSRs provide a reliable force measurement when calibrated but require extensive adjustment to produce absolute force values. Conversely, button

compression load cells also offer reliable force measurement (ICCs .998-1.00) as well as a linear response to applied force, although load cell data would benefit from a strict calibration process to increase accuracy. Therefore, from the present study findings the load cells will be chosen as the preferred bite force device, based on their linear response, high repeatability (with less 95% CI variation in comparison to FSRs) and suitability to provide immediate feedback on bite force, through the Delsys EMG system.

2.4 Can Masticatory Electromyography be Normalised to Submaximal Bite Force?

Introduction

Bite force and masticatory muscle activity can be used to assess the functional performance of the jaw within research studies, and may be of some use within clinical studies. Previous investigations have combined bite force measurements with electromyography (EMG) to explore differences in masticatory muscle symmetry (Ferrario et al., 2000), masticatory function of participants with different facial types (Gomes et al., 2010; Hara et al., 2010). Similarly, previous research has investigated the masticatory function of healthy individuals versus those with a limiting condition such as temporomandibular disorder or migraines (Burnett et al., 2000).

Clinical and research investigations using muscle activity and masticatory tasks have varied considerably in their approach to measuring and analysing EMG data. Not only do the recording systems, electrode parameters, and operational processes differ amongst EMG studies (Castroflorio et al., 2008), the techniques for processing and normalising EMG data in craniofacial and bite force research differs depending on the purpose of each investigation. For example, studies investigating chewing, speech, or other common submaximal tasks have tended to employ intercuspal Maximal Voluntary Contractions (MVCs) as a means of normalising EMG data (Gomes et al., 2010). Although this approach allows for experimental tasks to be

compared as a proportion of a maximal task, the MVC may underestimate true muscular contraction ability due to pain and/or discomfort experienced when attempting to generate 'maximal' bite force with bare dentition (Bakke, 2006). Studies investigating bite force and clenching have used a device or dampening material to produce the MVC. For example, Ferrario et al. (2000) asked participants to bite maximally on cotton rolls to normalise EMGs from intercuspal MVCs and clinical movements. In a later study the same group (Ferrario et al., 2004) acknowledged that normalising bite force to an action, whether it be maximal or submaximal, performed on a different surface to the experimental conditions, may incur a greater level of variability. These functional differences may be due to changes in muscle length at mouth opening height (Paphangkorakit and Osborn, 1997; Rues et al., 2008), the level of protection of occlusal surfaces to reduce discomfort (Bakke, 2006), or stability and position of bite force devices (Koc et al., 2010). Using a reference voluntary contraction, such as a nominal bite force level of 98N, to normalise EMG data from simple chewing tasks is one possible technique (Saifuddin et al., 2001). Employing reference voluntary contractions has become a commonly used practice in EMG studies when participants are unable or unwilling to perform MVCs (Healey et al., 2005; Burden, 2010).

Some previous studies have presented facial EMG data as absolute values rather than normalising them to a common EMG signal (Burnett et al., 2000; Ferrario et al., 2004; Tecco et al., 2007). However, the amount of variation due to differences in sex, skin thickness, and electrode placement can be reduced if the results are normalised. In the absence of normalisation, Kemsley et al. (2003) reported higher

EMG inter-volunteer versus intra-volunteer, and inter-session variation during chewing tasks. Thus, a well-controlled EMG protocol is fundamental to increase results reproducibility (Castroflorio et al., 2008).

Aims and Objectives

This study investigated the suitability of an alternative sub-maximal bite force normalisation process.

The objectives of the study were to:

(i) Evaluate whether facial muscle activity is linearly correlated with incremental sub-maximal and maximal bite force levels.

(ii) Assess whether normalising maximal and submaximal muscle activities to that produced when performing a low submaximal bite force level (i.e., 20N) improves between session experimental reliability.

Materials and Methods

This study used a total of thirty white Caucasian participants (15 males and 15 females, age range 18-25yrs, mean age 21.0 ±1.9yrs). All participants gave written informed consent before participating in the study. Participants completed a health questionnaire prior to taking part in the study (Appendix E), which detailed lifestyle factors, medical history and dental history. This was used as a screening tool, to

ensure participants met the inclusion criteria. The exclusion criteria for the study was a previous history of facial fracture or facial surgery, current or recent orthodontic treatment, any dental treatment within 6 months that consisted of more than a routine check-up and any medical investigations (particularly including x-ray or CT scanning). Additionally, any long-term parafunctional habits such as bruxism, temporomandibular dysfunction or masticatory pain, conditions or treatments that are known to effect musculoskeletal bone health, or pregnancy. Additionally, anyone who had a pacemaker fitted or were unable to lie on their front. The inclusion criteria for the study was white Caucasian aged between 18-25yrs, both males and females were recruited.

Reliability Measures

A participant sub-cohort (n=4 females) were invited to repeat the testing session 6 months later. All repeat participants complied with the previous inclusion criteria and none had undertaken any dental/surgical/medical procedures during the intervening period. Each participant followed the same protocol as previously detailed with no evident learning effect, due to the timeframe between sessions.

Figure 2.27: Surface EMG electrode placement for the Masseter and Anterior Temporalis.

EMG and Bite Force Equipment

Surface EMG electrodes (Delsys, Boston M.A., USA) were used to detect raw EMGs, the electrode placement was conducted as described in Section 2.2 (Figure 2.27). Bite force measurements were obtained using the custom made bite force device sandwich between disks of EVA (as previously described) to protect the participants dentition and secured with electrical tape. Each device (8.8mm in total height), was then inserted into a latex-free vinyl sleeve, which provided additional waterproofing for the load cell and the initial 40-50mm portion of the attached wire as shown in Figure 2.36.

EMG and Bite Force Protocol

During testing, participants sat comfortably, upright on a computer style chair facing a monitor positioned at eye level. EMGs were recorded at rest, maximal bite force and sub-maximal bite force. Firstly, 10s recordings were made during complete rest, gentle occlusion and occlusion with the bite force devices held between the upper and lower posterior dentition. The participants placed the bite force devices between their posterior teeth, where they felt most comfortable and were most able to bite evenly across the two devices. Participants were then asked to perform 3 repetitions of maximal voluntary biting (held for 2-3s during a 10s period) with adequate rest between repetitions. During this phase, the investigator provided positive encouragement whilst the participants viewed the feedback monitor, which displayed their bite force during the 10s recording window. The investigator noted down the maximum voluntary bite force (MVBF) from all repetitions and calculated 75%, 50% and 25% of this.

Subsequently, each participant completed three sub-maximal bite force recordings; a horizontal line target was placed on the feedback screen at 75%, 50% and 25% MVBF in turn. Each participant was instructed to clench for ~2s to reach the intended target and then to relax for ~2s repeatedly for a period of 20s to create a wave formation on the screen. Finally, each participant was asked to perform the submaximal clench-relax task, to a 20N bite force target. Once all the sub-maximal levels were recorded, the test was complete. Between participants the EVA and latex sleeve were discarded, the load cell was disinfected and a fresh covering applied.

Data Reduction and Analysis

Muscle activity and bite force data was analysed using the Delsys EMGWorks Analysis software (Delsys, Boston M.A., USA), which facilitated the analysis of all six channels (2 bite force, 4 muscle activity) simultaneously. The Root Mean Square (RMS) of each repetition was processed using a 0.3s moving window. The investigator identified the maximal and submaximal (75%, 50% & 25%) bite force values from within the data for both left and right sides independently, for each participant. These were selected within a 0.15-0.2s period, then exported to Microsoft Excel spreadsheet alongside the synchronised mean EMG values for all four muscles (L-R Temporalis & L-R Masseter).

Processed EMG data from each task was normalised by dividing it by muscle activity recorded during a 20N bite force. Initially, left and right bite force and muscle activity were normalised separately. Comparisons of left and right data using a t-test (SPSS statistical analysis software [IBM SPSS Statistics 19]) found no significant differences ($p > 0.05$) for bite force or muscle activity in either muscle. This was true of both males and females separately and pooled (Chapter 3: Table 3.1). This finding is concurrent with Van Der Bilt et al. (2008) who found no left-right differences in males and females from the ages of 19-69yrs ($p>0.05$). Therefore, the average activity across left and right sides, for each muscle, was calculated for all individuals. Levene's test for homogeneity (across all conditions) indicated equal variances ($p>0.05$) for all independent comparisons, therefore the assumption of homogeneity was met. Skewness and Kurtosis analysis indicated that the z-values for all variables

were within the ±1.96 range, therefore they did not differ significantly from normality. Mean normalised EMG data at every bite force level (25%, 50%, 75%, and Max), were plotted against the mean non-normalised data for each muscle, and grouped according to subject sex and muscle (see Figures 2.28-2.31). Pearson's correlation coefficients were calculated (SPSS statistical software) for each muscle across the four levels of bite force, subdivided by sex and normalisation.

Reliability Analysis

Individual and group Coefficients of Variation (CV) were calculated for non-normalised and normalised EMG data between both testing sessions using Equation 1:

$$CV = \frac{SD}{\bar{x}}$$

Equation 1: Coefficient of Variation where SD is standard deviation and $\bar{x}$ is the mean of the sample.

The average CV was calculated separately for the left and right sides, at each maximal and submaximal level, for both muscles. Furthermore, a two-way mixed model Intraclass Correlation Coefficient (ICC) was calculated with SPSS statistical software, using pooled left and right data, at each maximal and submaximal level for both muscles.

Results

Figures 2.28-2.31 show a linear relationship between jaw elevator muscle activity and incremental sub-maximal and maximal bite force levels, in both male and female cohorts.

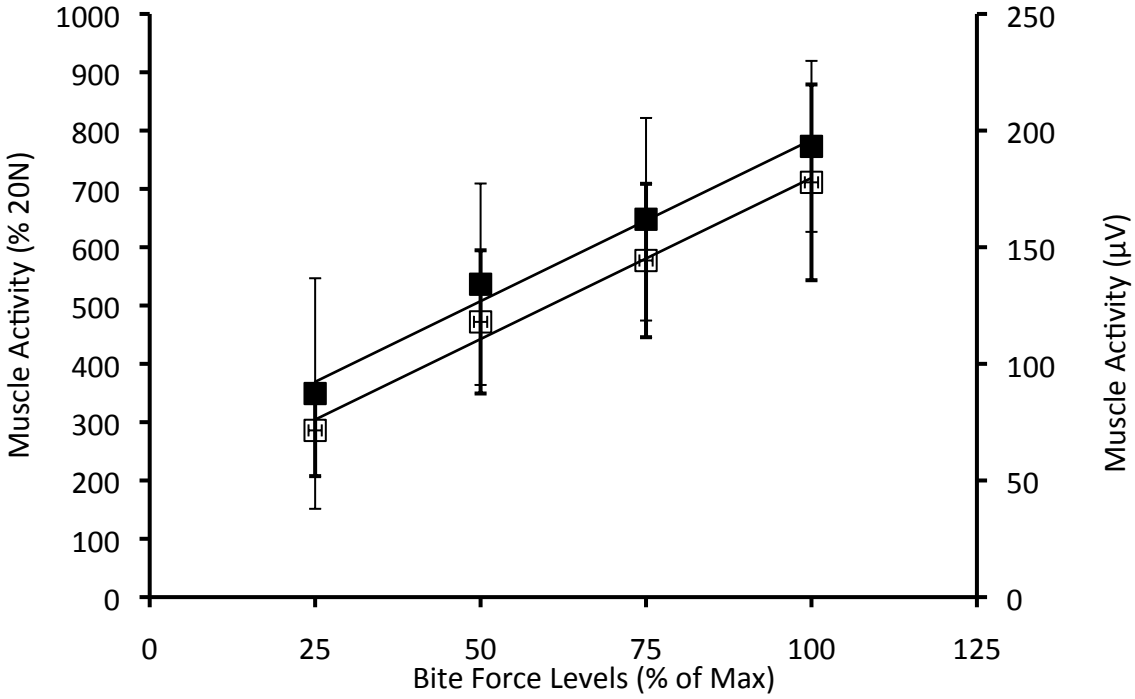


Figure 2.28: Masseter muscle activity at each bite force level (<25yrs Males).

Key: Mean (±SD) ☐ Masseter EMG normalised to 20N bite force (%); ■ Masseter non-normalised EMG values, (µV).

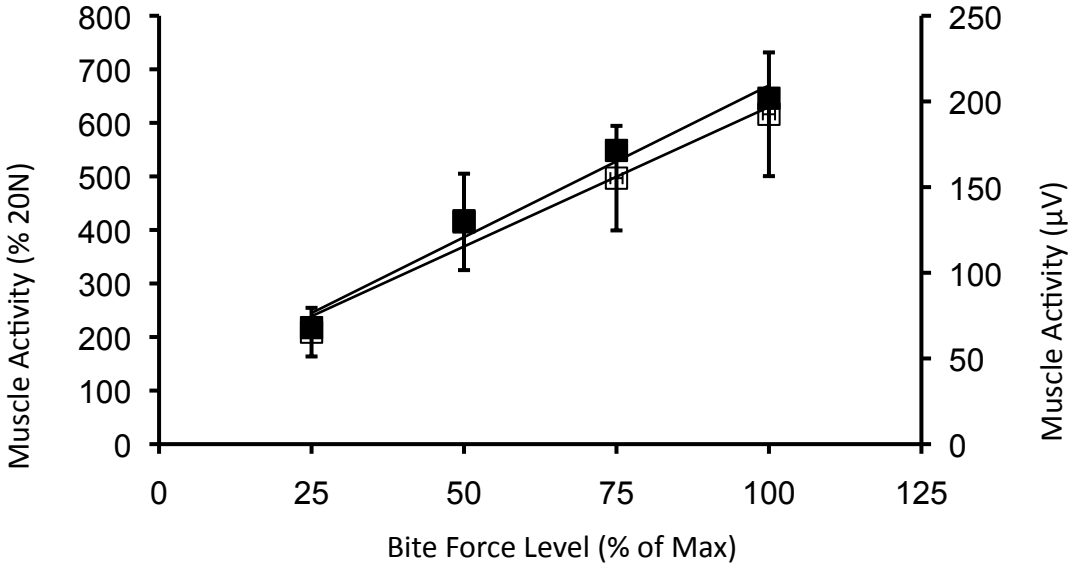


Figure 2.29: Masseter muscle activity at each bite force level (<25yrs Females).

Key: ☐ Masseter EMGs normalised to 20N bite force (%); ■ Masseter non-normalised EMG values (µV).

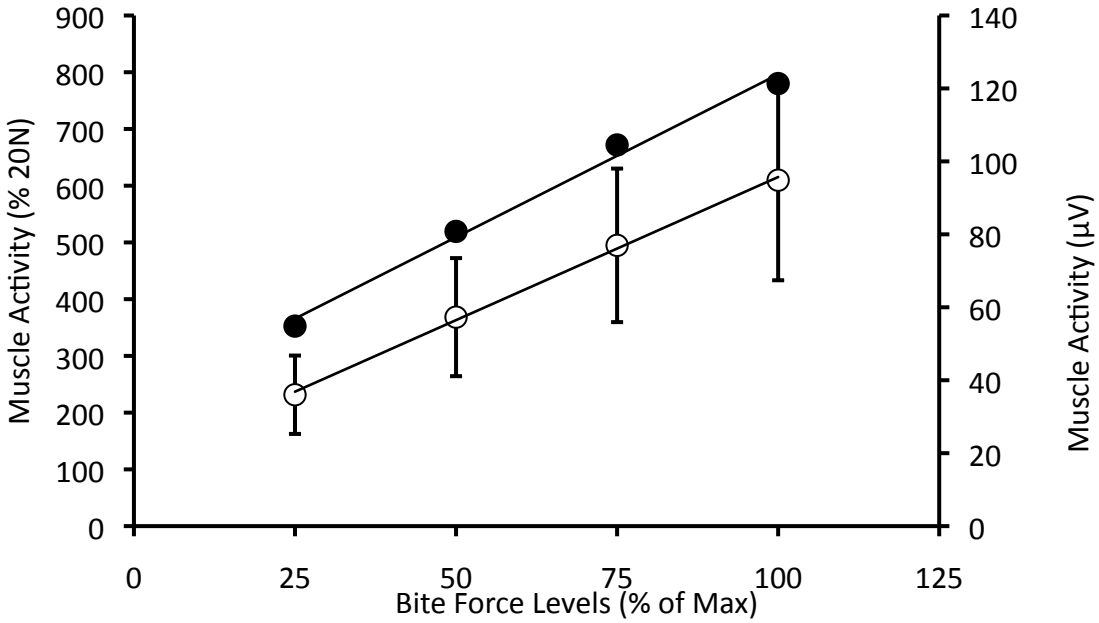


Figure 2.30: Temporalis muscle activity at each bite force level (<25yrs Males).

Key: Mean (±SD) ○ Temporalis EMG normalised to 20N bite force (%);● Temporalis non-normalised EMG values, (µV).

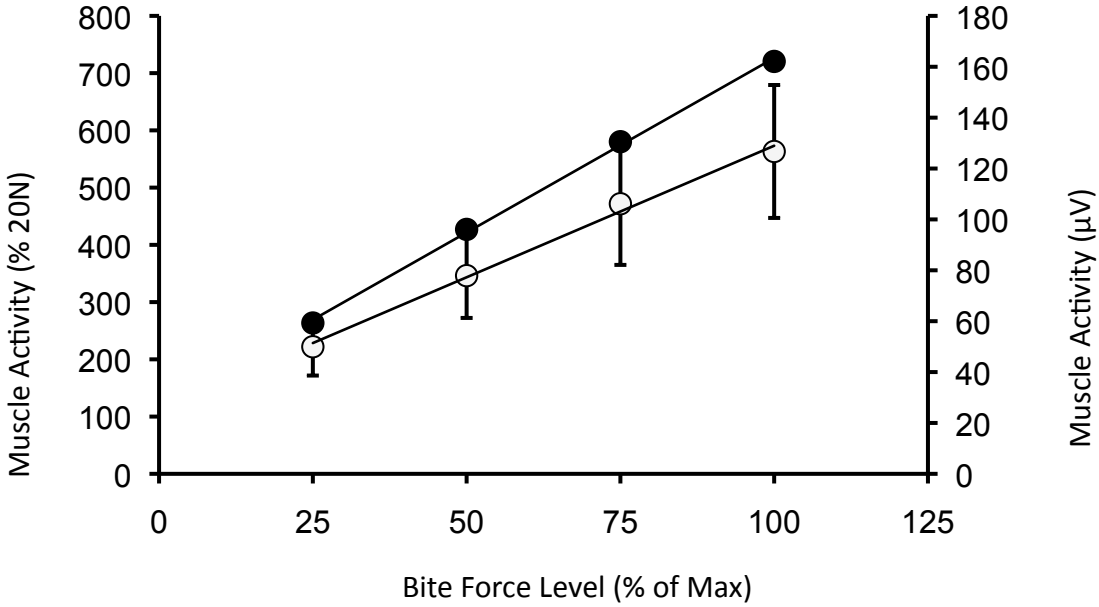


Figure 2.31: Temporalis muscle activity at each bite force level (<25yrs Females).

Key: ○ Temporalis EMG normalised to 20N bite force (%);● Temporalis non-normalised EMG values, (µV).

Despite small differences between normalised and non-normalised data, each data set had a high Pearson's correlation coefficient ($r>0.94$), indicating that the majority of muscle activity variance is attributable to the sequential changes in bite force.

Reliability Results

The between-session CVs (Table 2.3) for each muscle and level of bite force predominantly demonstrate reduced variance when the EMGs were normalised, with the only exception being the anterior temporalis muscle at 75% of maximal bite force.

Coefficient of Variation (%)					
Muscle	Bite Force Level	Left Non-Normalised	Left Normalised	Right Non-Normalised	Right Normalised
Masseter	Max	60.5	27.9	64.3	40.1
	75%	43.6	31.0	49.6	40.0
	50%	54.4	44.8	55.0	48.0
	25%	59.1	24.0	58.9	27.1
Anterior Temporalis	Max	51.3	39.4	80.1	48.2
	75%	50.2	59.7	55.5	47.4
	50%	56.1	52.0	66.0	58.9
	25%	55.7	32.2	74.5	53.1

Table 2.3: Coefficient of Variation (CV) for each muscle and bite force level.

Intraclass Correlation Coefficient			
Muscle	Bite Force Level	Non-Normalised	Normalised
Masseter	Max	-0.366	0.169
	75%	-1.171	0.342
	50%	-0.271	0.245
	25%	-0.187	0.698
Anterior Temporalis	Max	0.265	-0.409
	75%	-0.92	0.672
	50%	-1.115	0.434
	25%	0.29	0.566

Table 2.4: ICCs for each muscle and bite force level.

The ICCs (Table 2.4) for each muscle and level of bite force predominantly indicate an improved reliability between testing sessions when data are normalised, with the exception of the anterior temporalis muscle at maximum bite force.

Discussion

This study examined an alternative method for normalising EMG data recorded from the masticatory muscles during maximal and submaximal biting tasks. Facial muscle activity was expected to have a linear relationship with incremental sub-maximal bite force levels, up to maximal bite force, and that normalising EMG data to a low bite force (20N) would reduce the amount of variation between testing sessions.

First, the results showed (Figures 2.28-2.31) that facial muscle activity is linearly correlated with incremental submaximal and maximal bite force levels, across male and female groups and masticatory muscles. Pearson r-values were >0.94 for all comparisons, indicating very strong positive correlations between muscle activity and 25%, 50%, 75% and 100% bite force intervals. These findings are an improvement on previous work that found similar positive correlations up to 80% of maximal biting (Hosman and Naeije, 1979) and are similar to the findings of Ferrario et al. (2004) who reported a correlation >0.964 for bite force and submaximal EMG in healthy young participants.

The present study showed that normalising to a low bite force (20N) decreased the CV and increased the ICC in most comparisons. The majority of published bite force and EMG studies normalise to an MVC, as the focus of their investigations were submaximal tasks such as chewing (Gomes et al., 2010), clinical movements of the jaw (Siéssere et al., 2009), or biting at prescribed levels of force (Rues et al., 2008).

The present study used a low bite force (20N) reference value for normalisation, which allowed for successful normalisation from 25%-100% bite force. Other researchers have used MVCs performed on transducers or dynamometers to normalise an array of submaximal tasks on different biting (or non-biting) surfaces (Burnett et al., 2000; Siéssere et al., 2009). Although biting different surfaces may create greater variation in EMG or bite force results (Ferrario et al., 2000), normalisation is necessary for comparison of individual versus group results but also enables researchers to compare their results with prior studies (Hertel et al., 2004). The present study used the same bite force devices throughout all experimental tasks, therefore potential variability caused by mouth opening height and biting technique was reduced. Furthermore, through normalisation, these results are suitable for cross-group comparisons. Other studies using EMG have employed normalisation to bite force levels other than MVC for analysing everyday tasks. Burnett et al. (2007) found that normalising posterior and posterolateral neck muscles to 60% MVC was reliable for both surface and intramuscular EMG electrodes. Similarly, in non-facial EMG, Healey et al. (2005) normalised paraspinal muscle activity in participants suffering from chronic lower-back pain, to a reference voluntary contraction obtained whilst they held a weight outstretched. Although the reference contraction was recorded during a different movement to the experimental conditions, it prevented any additional pain or discomfort to the participants that may have been caused by performing a MVC. Saifuddin et al. (2001) measured EMG of the masticatory muscles during daily tasks such as chewing gum, sleeping, and eating a meal. Similar to the present study, they normalised muscle

activity to a chosen submaximal level (produced during a 98N bite force), which reduced mealtime EMG variation between sessions.

The current study used CVs and ICCs to quantify the reliability of the normalisation process. The CVs markedly decreased when EMG data were normalised across both muscles and all bite force levels, with one exception highlighted in Table 2.3. Moreover, the ICC results improved dramatically when EMG data were normalised. Studies that have measured EMG data reliability, regardless of anatomical position, have employed a number of statistical analyses to quantify the repeatability of the measurement technique: ICCs, CVs, standard error of measurement, and repeated measures analysis of variance are commonly used (Knutson et al., 1994; Worrell et al., 1998) Weir, 2005; Minshull et al., 2009). Burdette and Gale (1990) reported between-session reliability (Pearson's r-values) for the masseter muscle ranging between 0.56-0.65 and 0.33-0.48 for the temporalis. Although they used interclass rather than intraclass correlation statistics for reliability measures, which is not recommended for a repeated measures analysis, their results indicated increased variability within the anterior temporalis versus the masseter muscle, which they attributed to the temporalis' role in maintaining mandibular postural rest. Greater variability found in this and the present study may indicate innate differences in muscle activity between the elevator muscles. Suvinen et al. (2009) presented between-day ICC values for the masseter and anterior temporalis muscles during mouth opening and closing tasks, with ICC results ranged from 0.877-0.899 during clenching. The present study observed a greater ICC range and lower ICC values

(0.16-0.69) compared to Suvinen et al. (2009), which could be explained by differences in EMG equipment and placement.

In conclusion, normalising EMG data to a submaximal bite force level of 20N, highlighted a linear relationship of jaw elevator muscle activity with sub- and – maximal bite force levels. Normalisation successfully improved between-session reliability in comparison to non-normalised data. The prescribed low bite force of 20N will facilitate inter-group comparisons and reduce natural variations in masticatory muscle activity. This will prove particularly useful when studying bite force/EMG relationships in patients with musculoskeletal conditions or in ageing populations. This study shows that normalising EMG values to a reference level other than MVC, a technique that has been used in other disciplines that utilise EMG, can be successfully applied to dental and craniofacial research with good effect.

This approach will be carried out throughout the thesis, for the benefit of the older cohort during testing and to allow for comparisons to be drawn between the older and younger age groups. This will therefore facilitate investigation of the muscle – bone – bite force relationship across different sexs, age groups and ethnicities, and in the most relevant cohorts.

2.5 Measurement of Bone Mineral Density

Bone mineral density has been measured using dual energy X-ray absorptiometry (DXA) extensively throughout research, it is not the only measuring tool but it is the most widely used (Adams, 2013). DXA uses two X-ray beams operating at different energy levels, which are emitted from a radiation source and aimed at a radiation detector, the participant (or participant's body part) is positioned between the source and the detector (El Maghraoui and Roux, 2008). However, there is a propensity for error in high adipose tissue areas, because the DXA uses two X-ray energies in the presence of three types of tissue (bone, lean tissue and adipose tissue), the BMD data can become less accurate (El Maghraoui and Roux, 2008). Unlike conventional radiography, DXA emits low levels of radiation (forearm, 0.5 µSv; spine, 2-4 µSv; femur, 2-5 µSv per scan), which are less than the average natural back ground radiation (~7 µSv/day) (Adams and Bishop, 2008). This low exposure to radiation recommends DXA as a suitable tool for bone health diagnosis and research Adams, 2013). Consequently, DXA is commonly used for clinical diagnoses of bone health disorders such as osteopenia and osteoporosis (El Maghraoui and Roux, 2008) and is used to measure body composition, particularly in athlete sample groups, within sports science research. The measurement of the hip, lumbar spine and distal radius, are key skeletal sites used in clinical investigations (Adams, 2013). The World Health Organisation (WHO) use a system which defines bone health disorders as 1 or 2.5 standard deviations below the young adult mean, known as the T-score value. A

threshold of ≤-2.5 determines osteoporosis, between -2.5 and -1.0 for osteopenia and ≥-1.0 for normal healthy individuals.

Further expansion of DXA as a research tool includes techniques for measuring BMD of the facial bones (Horner and Devlin, 1998), specifically in relation to key skeletal sites as an additional marker of bone health and osteoporosis (Drage et al., 2007; Horner et al., 1996). The mandible in particular has been measured and analysed successfully using DXA (Horner et al., 1996; Drage et al., 2007; Devlin and Horner, 2007; Esfahanizadeh et al., 2013). Due to a lack of specialist software to analyse the facial BMD, previous studies have opted to select regions of interest (ROIs) (Devlin and Horner, 2007) from a forearm scan defined by the manufacturer (Horner et al., 1996; Drage et al., 2007; Esfahanizadeh et al., 2013). This technique has also been successfully used in dental radiographs, which were found to be fairly accurate diagnostic tools (Nackaerts et al., 2008; Geraets et al., 2014). With regards to DXA, this technique has provided suitable BMD data for comparison between the jaw and other skeletal sites in edentulous healthy and osteoporotic individuals. The awkward positioning for participants due to the location of the scan window on the DXA bed is one limitation of this technique (Drage et al., 2007). However, authors have developed ways of positioning participants (often with the use of a foam wedge), that allows for superimposition of the mandible and a reasonably comfortable lying position (Horner et al., 1996; Devlin and Horner, 2007; Drage et al., 2007; Esfahanizadeh et al., 2013). Most of these studies have examined older participants in relation to osteoporosis; the present study will measure a cohort of healthy 50+yrs using similar techniques. The measurement of healthy individuals will allow

comparisons to be made between key skeletal sites used to define bone health, and the mandible, which could address whether the mandible is a good predictor of bone health in non-osteoporotic cohorts. Furthermore, the presence of lower BMD in the mandible could help to identify individuals who are at greater risk of facial sports injury.

2.6 Materials and Methods

Health Questionnaire & Screening Protocol,

Participants completed a health questionnaire prior to taking part in the study, which detailed lifestyle factors, medical history and dental history (Appendix E). This was used as a screening tool, to ensure participants met the inclusion criteria for the study (as described in Section 2.4).

Facial Measurement Techniques

Jaw measurements were taken using callipers (Mitutoyo, UK) specially adapted for measuring the human body, placed on the Condyle at the region of the temporomandibular joint. This distance was measured twice with a mean value calculated. In addition, a profile photograph of each participant (taken of the right hand sagittal view) was obtained for the analysis of facial dimensions. Care was taken to ensure the participant was seated upright with the Frankfort plane horizontal to the floor and the jaw held in gentle occlusion. The facial structure was then analysed using 'ImageJ' photo analysis software. The measurements consisted of the distance from Condyle to Angle (Po – Go), Angle to Menton (Go – Me) and facial height (upper N – ANS and lower ANS – Gn) as shown on Figure 2.32.

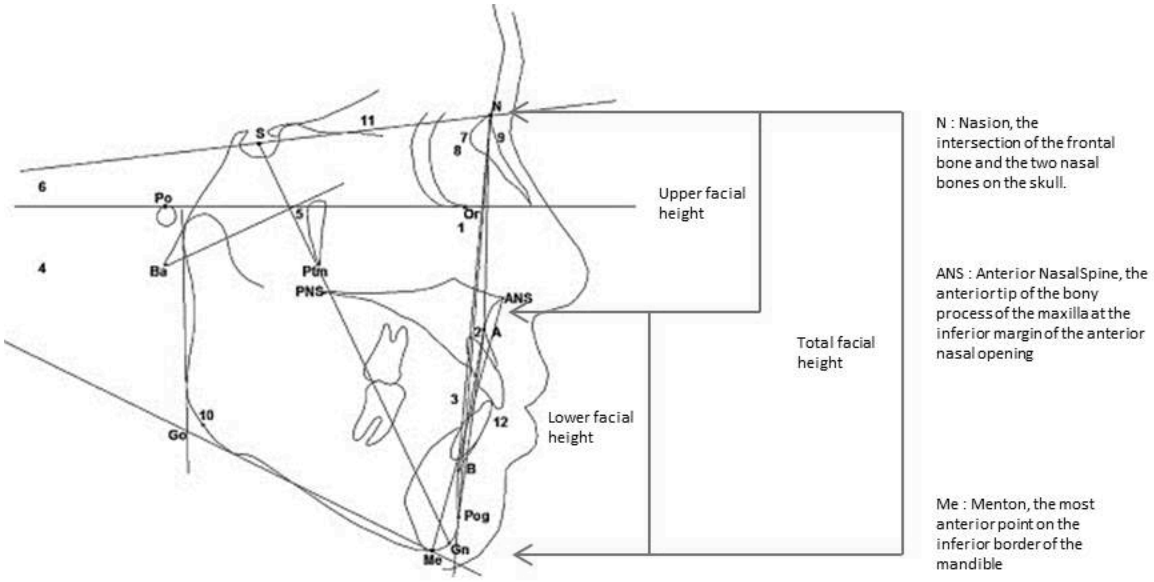


Figure 2.32: Measurement points for facial height, mandibular length and angle

(Original diagram obtained from Abbassy et al. (2012))

EMG and Bite force Equipment

Surface EMG electrodes (as described in Section 2.2) were used to measure muscle activity. The sensors were placed bilaterally over the main portion of the Masseter and on the anterior portion of the Temporalis (Figures 2.33-2.35). Prior to attaching the electrodes, the areas of skin were shaved if necessary and cleaned with an alcohol wipe. The positioning of the electrodes was checked through palpation during clenching.

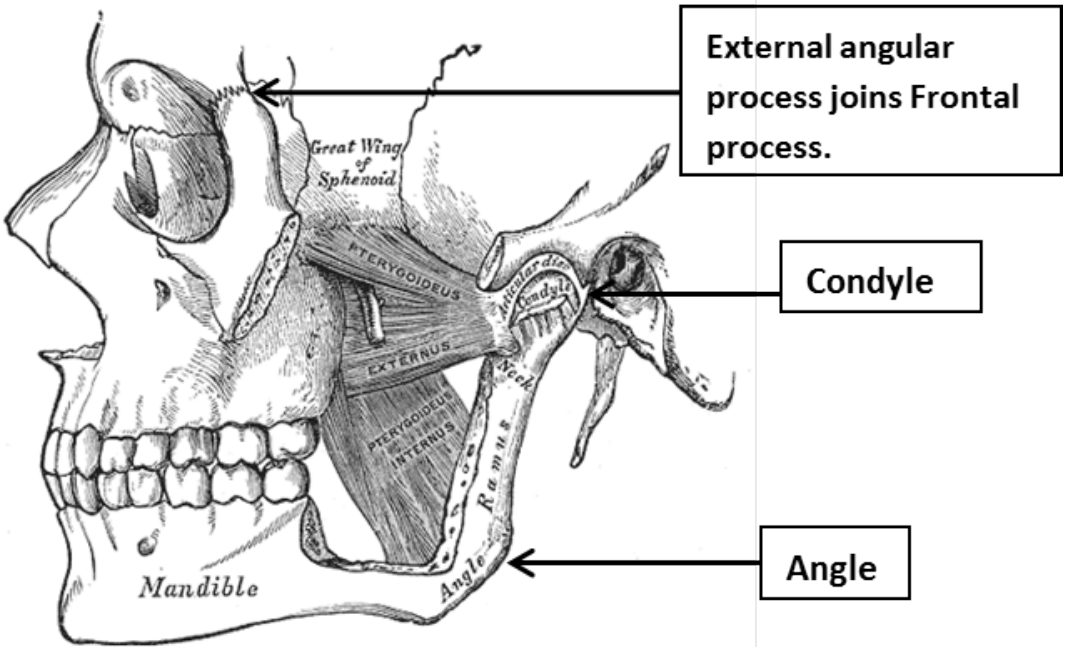


Figure 2.33: Skeletal positioning of the Condyle and Angle of the Mandible.

(Original diagram obtained from Gray (2010)).

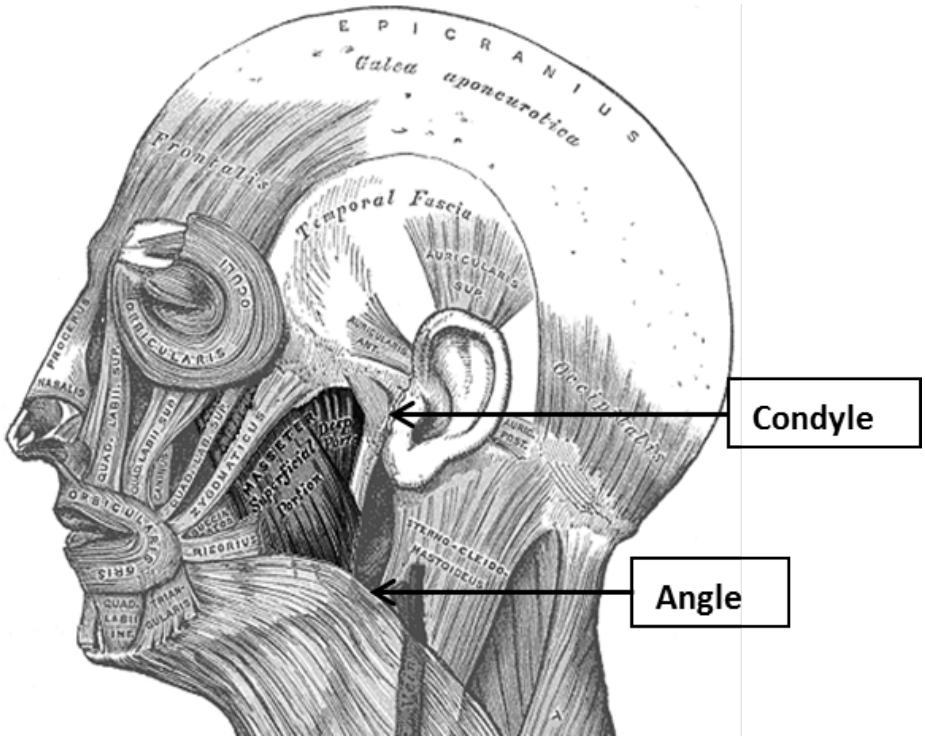


Figure 2.34: Positioning of the Condyle and Angle of the Mandible in relation to the jaw elevator muscles.

(Original diagram obtained from Gray (2010).

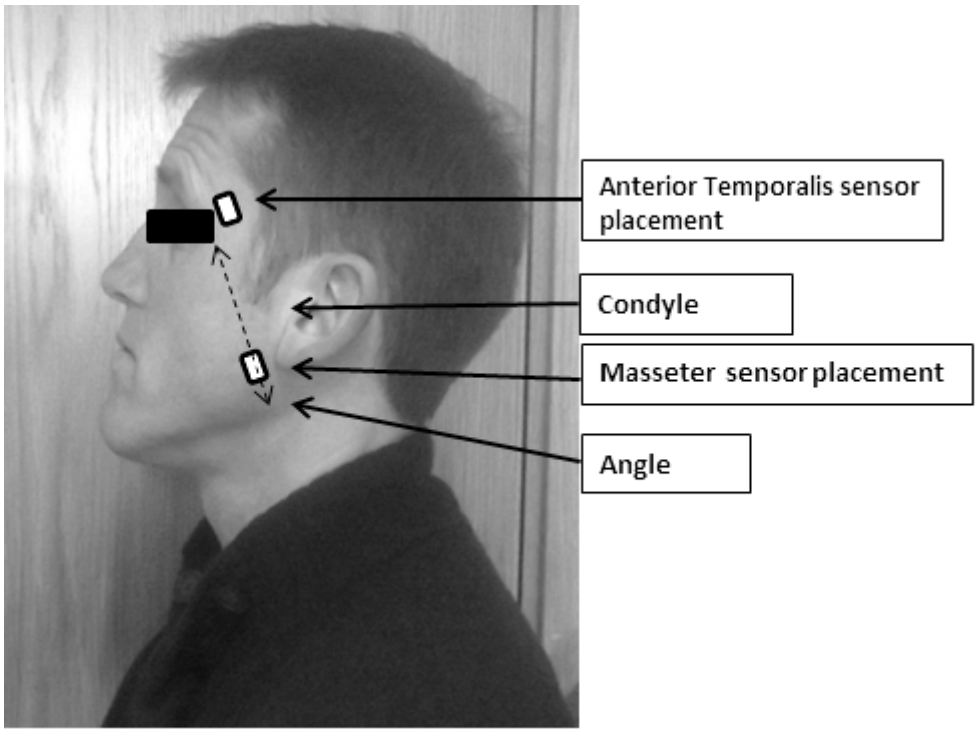


Figure 2.35: Positioning of the Condyle and Angle of the Mandible *in vivo* assessment.

Bite force measurements were obtained using the custom made bite force device (as described in Section 2.3 and 2.4) which were then connected to a Delsys Bagnoli 8-Channel EMG system through a bespoke amplifier box. Prior to testing, the bite force devices were calibrated using a LRX plus Materials Testing Machine (Lloyd Instruments Ltd., Hampshire, Uk).

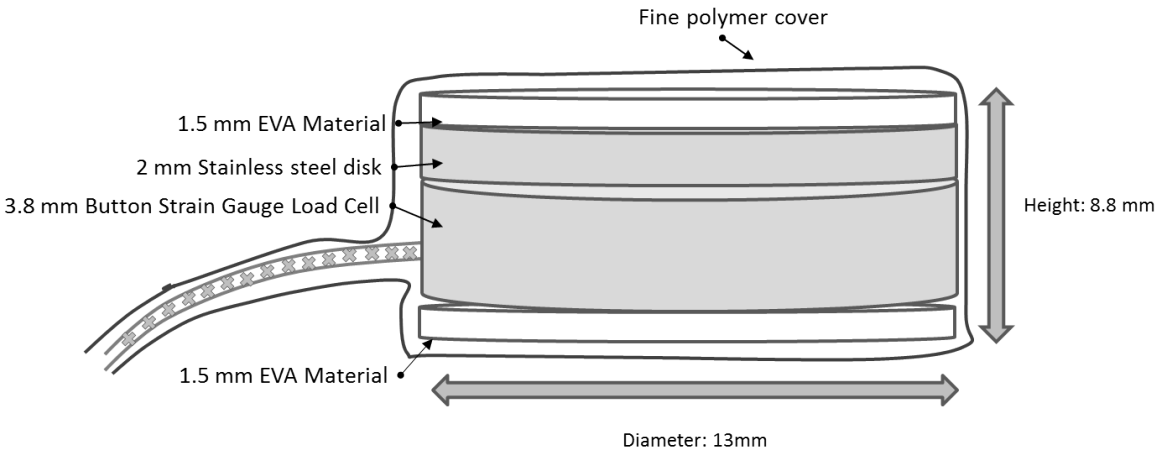


Figure 2.36: Novel Bite force device construction.

EMG and Bite force Protocol

The EMG and bite force protocol is described in full in Section 2.4.

BMD measurements

Bone mineral density values (g/cm^2) were obtained using a Discovery QDR dual energy X-ray absorptiometry (DXA) scanner (Hologic Inc, USA). Daily calibration scans were conducted on the QDR scanner during the experimental period, to ensure accurate values were obtained. Lumbar spine and femoral neck values were obtained using the respective scanning protocols on the accompanying software (Hologic Inc, USA). Each protocol consisted of a rectilinear window, which passed along the length of the spine or proximal end of the femur, as the participant lay supine on the bed (see Figures 2.37 and 2.38). The mandible BMD values were obtained using the 'Forearm scan software' a rectilinear window, extended to 18cm

in length. As there is no specific software in the DXA scanning equipment designed to measure and analyse facial/mandibular BMD, studies have previously used the forearm scan software (Horner et al., 1996; Adams, 2013; Esfahanizadeh et al., 2013). The present study follows a similar protocol to those previously described. Drage et al. (2007), Esfahanizadeh et al. (2013) and Horner et al. (1996) all place the participants semi-prone with their head supported on a wedge, which was reportedly uncomfortable for participants to maintain for the duration of the scan (Horner et al., 1996). In the present study the participants were asked to lie prone on a physiotherapy bed, which was placed perpendicular to the DXA bed as shown in Figure 2.39. They were asked to turn their face so that one side of the mandible lay flat on the bed, with the opposite side of the mandible superimposed on top as described in Horner et al. (1996), this was to ensure bone measurements did not include roots or full dentition. In the event of a poorly superimposed scan, the process was repeated for that side. The face scan was performed on both sides, to assess repeatability of the mandibular scan. The Lumbar spine BMD was calculated as a mean of L1-4 (g/cm^2) and femoral neck BMD was calculated using a $2.5cm^2$ rectangular window (see Figure 2.40), positioned along the femoral neck width. The mandible BMD was calculated as a mean of 4 x $0.22cm^2$ analysis windows, at both the angle of the ramus and lateral edge of the mandible.

Figure 2.37: Position of the participant during the dual-hip scan.

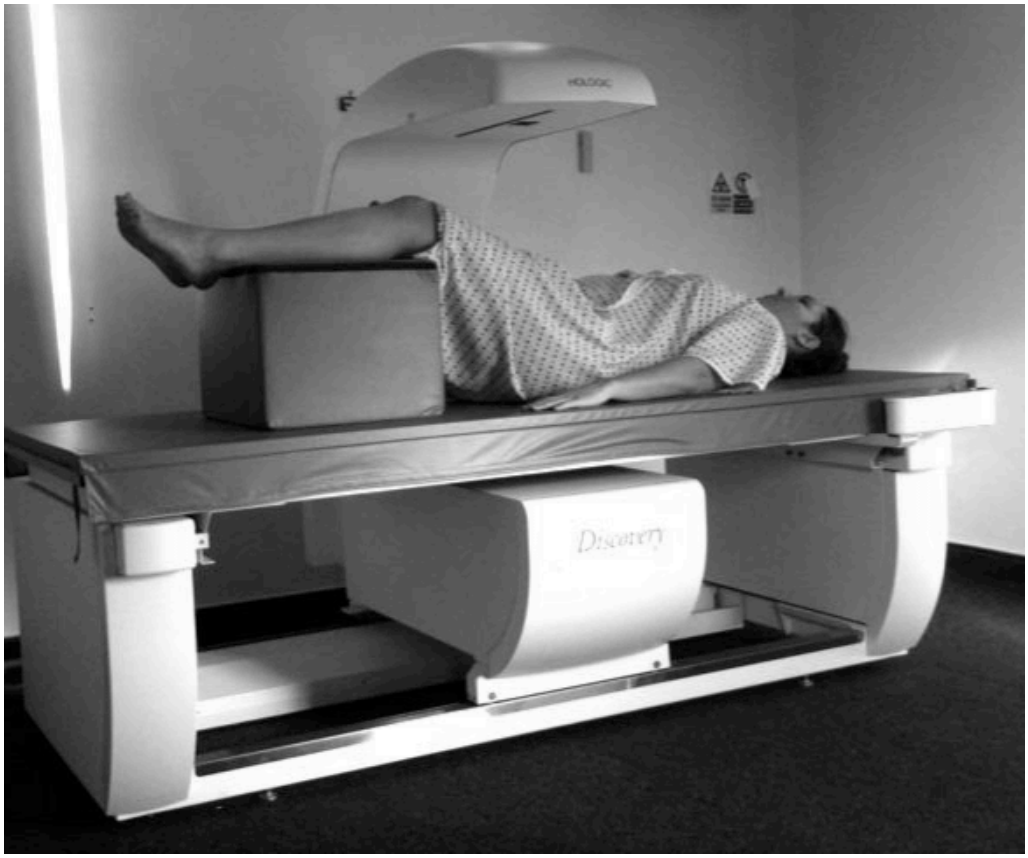


Figure 2.38: Position of the participant during the lumbar spine scan.

Figure 2.39: Position of the participant during the mandible scan.

Data Reduction and Analysis

EMG and Bite Force

Muscle activity and bite force data was analysed using the Delsys EMGWorks Analysis software (Delsys, Boston M.A., USA), which facilitated the analysis of all six channels (2 bite force, 4 muscle activity) simultaneously. The Root Mean Square (RMS) of each repetition was processed using a 0.3s moving window. The

investigator identified the maximal and submaximal (75%, 50% & 25%) bite force values from within the data for both left and right sides independently, for each participant. These were selected within a 0.15-0.2s period, then exported to Microsoft Excel spreadsheet alongside the synchronised mean EMG values for all four muscles (L-R Temporalis & L-R Masseter).

Processed EMG data from each task was normalised by dividing it by muscle activity recorded during a 20N bite force. This was to allow comparison of muscle activity during maximal bite forces, and to aid comparison of younger participants with elderly participants, who may find performing maximal bite force tasks uncomfortable. Initially, left and right bite force and muscle activity were normalised separately. Comparisons of left and right data using a t-test (SPSS statistical analysis software [IBM SPSS Statistics 19]) found no significant differences ($p > 0.05$) for bite force or muscle activity in either muscle. This was true of Males and Females across all sample groups. This finding is concurrent with (Van Der Bilt et al., 2008) who found no left-right differences in males and females from the ages of 19-69yrs ($p>0.05$). Therefore, the average activity across left and right sides, for each muscle, was calculated for all individuals. Additionally, no significant differences were found between left and right femoral neck and facial sites BMD, using the same t-test comparison of means. Owing to this, the mean of left and right of all skeletal sites was calculated for all individuals.

BMD Analysis

Mandible BMD was measured bilaterally using the forearm scan software, and regions of interests (ROI's) were created manually by placing 4 x 0.22cm^2 analysis windows at both the angle of the ramus, and along the lateral edge of the mandibular body. This method is similar to others previously used in facial BMD studies (Horner et al., 1996; Drage et al., 2007), except that the regions of interest (ROIs) were of a uniform but much smaller size for every participant, and a mean of four ROIs was calculated to obtain one BMD value for both facial sites. Additionally, care was taken to avoid the roots of the dentition when measuring the mandibular body, as this can give false BMD values. Additionally, no significant differences were found between left and right femoral neck and facial sites BMD, using the same t-test comparison of means. Owing to this, the mean of left and right of all skeletal sites was calculated for all individuals.

Figure 2.40: ROI used to measure femoral neck BMD.

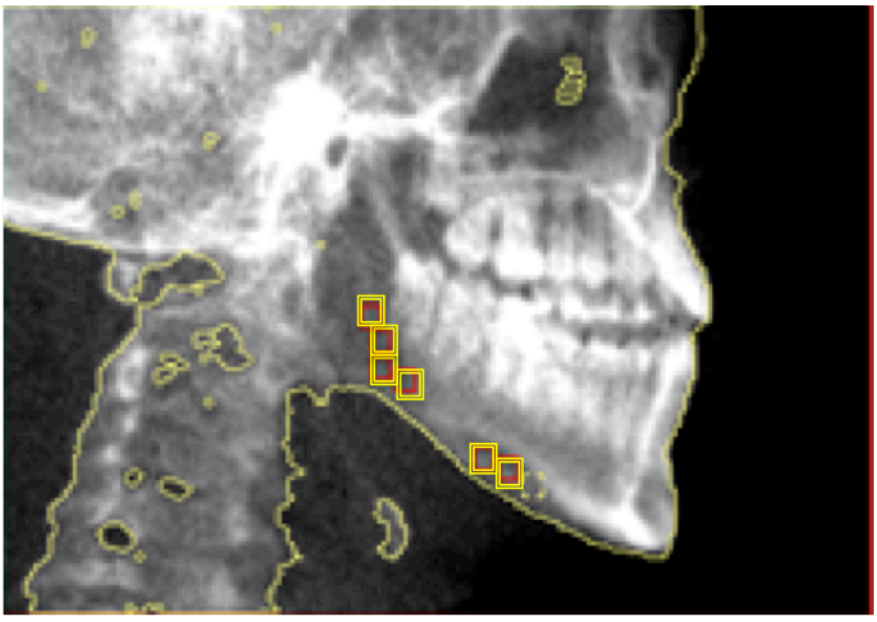


Figure 2.41: ROIs used to calculate mandibular BMD at the ramus and mandibular body.

These methods will be carried out throughout the experimental studies Chapters 3-6; the adapted protocols in EMG normalisation and BMD scanning have been adopted for the benefit of the older cohort during testing and to allow comparisons to be drawn between the older and younger age groups. This will therefore facilitate a standardised investigation of the muscle – bone – bite force relationship across sexs, age groups and ethnicities. The following chapter will examine bite force, muscle activity and mandibular bone mineral density between young adult males and females.

Chapter 3: The effect of sex on the bite force, muscle activity and BMD of young Caucasian adults.

Introduction

Musculoskeletal differences

Differences in physical characteristics, such as average height, weight, muscle mass and muscle strength, between males and females are maintained throughout adult life (Barlett et al., 1991; Goodpaster et al., 2006). Barlett et al. (1991) reported that fat free mass: height ratio showed no significant sex difference in children and early adolescents but became significantly ($p=0.0001$) different at 16-18yrs and remained so throughout adulthood. On average males tend to be taller and heavier than women (Riggs et al., 2004) with a greater amount of fat free mass (Barlett et al., 1991), and higher bone turnover, even after correcting for bone size, which may have implications on the skeletal response to strain and impact exercise (Henry and Eastell, 2000). Furthermore, sex has been found to affect the force capabilities of upper and lower limb muscles. In a cohort aged 45-78yrs Hughes et al. (2001) reported that males had consistently and significantly ($p<0.001$) higher muscle strength than females in the elbow flexors (45±10Nm males, 22±7Nm females) and elbow extensors (45±12Nm males, 27±9Nm females), knee flexors (92±21Nm males, 54±10Nm females) and knee extensors (160±29 males, 98±20 females). In addition Raadsheer et al. (2004) reported a significant link between limb muscle thickness and jaw muscle thickness in healthy young adults (57 males and 64 females, mean age 23yrs), however this link was not replicated in limb and jaw muscle force. The

study showed that muscle forces were significantly different between males and females, at upper and lower limbs (right arm flexion 383.8±61.7N males, 213.0±42.8N females, right leg extension 645.7±143.7N males, 389.3±87.8N females), as well as the jaw (bite force 547.2±115.6N males, 383.3±86.3N females). These findings indicate a pre-existing sex difference in force production and muscularity across skeletal sites, but they also indicate that muscularity and bite force are not necessarily connected to limb force and muscularity. Sexual dimorphism in muscular strength may have further implications on the muscle-bone relationship, at loaded skeletal sites and in the craniofacial skeleton.

Bone Mineral Density

Bachrach et al. (1999) reported that sex differences in bone mass are sufficient to justify the use of sex specific reference data, when interpreting paediatric and young adult bone mineral status using DXA, particularly in clinical research. However, significant differences in BMD between sexs appears to be largely attributable to greater bone size in males, as Henry and Eastell (2000) reported greater BMD in females when corrected for bone size at the lumbar spine and total body, in a group of young Caucasian adults aged 26yrs. These findings were corroborated by Peacock et al. (2009) who found total femoral neck and cortical volumetric BMD to be higher in Caucasian women (aged 23-57yrs) than Caucasian men (aged 20-63 yrs) throughout the age range, but cancellous volumetric BMD was not higher in women. The material and structural variables of whole bones has been under explored in

relation to adult sex differences (Seeman 2008a), but they may be key to significant differences between male and female BMD. Furthermore, the discrepancy in BMD measurements between males and females when correcting for or not correcting for bone size, suggests that higher fracture rates in females may be due to smaller bone size rather than lower BMD (Henry and Eastell, 2000; Looker et al., 2009). Lumbar spine and femoral neck BMD is affected by height, fat free mass and fat mass in older adults (aged 60-79 yrs) of both sexs (Gouveia et al., 2012). In contrast to Peacock et al. (2009), Looker et al. (2009) reported men over 50yrs had significantly higher BMD than women over 50yrs, across whole body, lumbar spine and pelvis sites, after correcting for bone size (specifically leg length). This may be due to the age related and post-menopausal changes to BMD and bone geometry experienced by females over 50yrs. The ability to correct for bone size was further scrutinised by Looker et al. (2009) suggesting that the variety of anatomical differences may be too many to consider when correcting BMD. Correcting BMD (g/cm^2) for leg length, still results in a 2 dimensional measurement of BMD, which is not as thorough as a 3 dimensional DXA scan or other form of scan such as MRI or pQCT.

Bite Force

Within dental research, the influence of sex on bite force and muscle activity has also fielded mixed results (Koc et al., 2010). Studies that have separated males and females, have reported significantly greater bite force values for males; Van Der Bilt et al. (2008) reported a bilateral bite force of 652±151N for males (mean age

37±16yrs) and 553±170N for females (mean age 39±14yrs), which is an 18% difference. Similarly, Varga et al. (2011) reported 777 ±78N for males and 481 ±190N for females aged 18yrs, which is a 62% difference. Conversely, other studies have reported no sex differences and therefore pooled male and female data (Ferrario et al., 2000; Thompson et al., 2001). Sex has been found to have a significant effect on bite force throughout life, with males producing significantly higher forces than females from teenagers (405±30N males vs 280±31N females aged 13-20yrs), through to 80 year olds (372±49N males vs 162±46N females aged 61-80yrs) (Palinkas et al., 2010). However, other findings have reported no significant difference in bite force between young adult males (383±102N) and females (338±113 N) aged 22-32yrs (Lepley et al., 2011).

Studies have reported the debilitating effect of medical conditions on bite force and muscle activity. Participants with conditions such as osteoporosis, temporomandibular disorder or cranio-mandibular disorders have been compared to healthy populations of males and females (Visser et al., 1994; Tortopidis et al., 1999; Pereira-Cenci et al., 2007; Siéssere et al., 2009), but some studies lack a balance and age- or –sex matched controls (Suvinen and Kemppainen, 2007). However, there is a need for further investigation of bite force and muscle activity within healthy populations (Ferrario et al., 1993; Suvinen and Kemppainen, 2007), in order to ascertain pre-existing differences in healthy sample groups, before comparing the effects of debilitating conditions. Thus, the status of bone mineral density in a younger adult population may be beneficial to examine, especially in relation to injury modality.

Young men in their late teens and early adulthood are the most prevalent hospital admissions for sports related injuries (Conn et al., 2003; Delilbasi et al., 2004; Mourouzis and Koumoura, 2005; Elhammali et al., 2010). The highest reported male to female ratio of injury rates is 19:1 (Delilbasi et al., 2004) but 9:1 (Mourouzis and Koumoura, 2005) and 8:1 (Roccia et al., 2008) are also reported in European studies, indicating a high proportion of young male casualties. However, these findings may be influenced by the ratio of male to female participation in sport, which is reportedly lower amongst teenage girls (78-87% lower) (Vilhjalmsson and Kristjansdottir, 2003) and young adult women (Telama et al., 2005). Nevertheless, for girls and women that do participate in sport, the fluctuation in hormones due to age at menarche and menstrual cycle length (Cooper and Sandler, 1997) may hinder BMD gain. In addition, the use of some forms of contraception have been found to negatively affect BMD in women (Berenson et al., 2004) and further negative fluctuations in BMD may be experienced during pregnancy (More et al., 2001). Therefore, female athletes, especially those partaking in high impact – low body weight sports such as gymnastics and triathlon, who experience unbalanced hormones and irregular menstruation, may be at greater risk of injury in comparison to their males counterparts.

Aims and Objectives

The aim of the present study was to investigate the effect of sex on bite force, muscle activity and mandibular BMD, in a population of healthy young Caucasian adults aged 18-25 years old. The objectives of the study were to;

(i) Identify how bite force, facial muscle activity and BMD relate to anatomical differences in sex.

(ii) Identify whether sex affects the bite force, jaw elevator muscle activity and BMD at the ramus and mandibular body.

(iii) Identify how the bite force, facial muscle activity and BMD correlated within each sample group.

Materials and Methods

Participants

The Department of Exercise and Sport Science Ethics committee, Manchester Metropolitan University, granted ethical approval prior to the study commencing. The exclusion criteria for the study was a previous history of facial fracture or facial surgery, current or recent orthodontic treatment, any dental treatment within 6 months that consisted of more than a routine check-up and any medical investigations (particularly including X-ray or CT scanning). Additionally, any long-term parafunctional habits such as bruxism, temporomandibular dysfunction or masticatory pain, conditions or treatments that are known to effect musculoskeletal

bone health, or pregnancy. A total of 30 Caucasian participants were recruited, which consisted of an overall experimental group of 15 males (mean age 20.9 ±1.71yrs) and 15 females (mean age 21.3±2.0yrs) aged 18-25yrs (whole group mean age 21±1.85yrs). All participants gave written informed consent prior to taking part in the study. The 18-25 year age range was chosen because tooth loss in healthy individuals significantly increases in the third to fourth decade of life (Broadbent et al., 2006), which can affect bite force (Bakke, 2006) and area specific material property changes in bone (Dechow et al., 2010). Furthermore, the greatest prevalence of sports injuries are sustained by young adults (Delilbasi et al., 2004; Mourouzis and Koumoura, 2005; Elhammali et al., 2010), which identifies them as one of the most at risk groups for facial injury. Details related to the bite force, muscle activity and BMD testing procedure are described in Chapter 2 Section 2.6 'Materials and Methods'.

Data Analysis

Firstly, the differences between left and right bite force, muscle activity and BMD values for the femoral neck, ramus and mandibular body, were compared using a t-test (SPSS statistical analysis software [IBM SPSS Statistics 19]). Group means for age, height and mass were compared using separate t-tests. One-way MANOVAs were performed on the bite force, muscle activity and bone mineral density data to ascertain the effect of height and mass. Further multifactorial analyses measured the effect of sex on four grouped dependent variables. The significance level was set to

$p < 0.05$ and the univariate significance level was set to $p < 0.0125$ using the Bonferroni correction for four dependent variables and $p < 0.01$ for five dependent variables (Field, 2009). Levene's test for homogeneity (across all conditions) indicated equal variances ($p > 0.05$) for all independent comparisons, therefore the assumption of homogeneity was met. Skewness and Kurtosis analysis indicated that the z-values for all variables were within the ±1.96 range, therefore they did not differ significantly from normality. Furthermore, Pearson's correlation coefficients were calculated between the main dependent variables (maximum bite force, maximum masseter muscle activity, maximum anterior temporalis muscle activity, BMD at the ramus and BMD at the mandibular body) as well as the four BMD measurement sites; ramus, mandibular body, femoral neck and lumbar spine.

Results

Bite force, muscle activity and BMD values for the femoral neck, ramus and mandibular body, showed no significant differences ($p > 0.05$) between left and right sides (Table 3.1.). Therefore, the averaged values for left and right bite force, muscle activity and BMD were used for further analyses. This was true of both the male and female sample group.

Males	Left (mean ±SD)	Right (mean ±SD)	p value
Bite force (N)	286.3 (±159.5)	304.2 (±141.8)	0.746
Max masseter (%)	648.8 (±438.1)	773.7 (±439.7)	0.442
Max temporalis (%)	558.8 (±436.2)	660.7 (±488.8)	0.552
Femoral neck (g/cm^2)	1.15 (±0.41)	1.13 (±0.27)	0.846
Mandibular ramus (g/cm^2)	0.62 (±0.30)	0.68 (±0.21)	0.581
Mandibular body (g/cm^2)	1.32 (±0.40)	1.47 (±0.73)	0.262
Females			
Bite force (N)	256.9 (±99.10)	276.0 (±112.4)	0.625
Max masseter (%)	653.0 (±289.8)	579.1 (±291.4)	0.492
Max temporalis (%)	520.2 (±241.0)	606.1 (±335.9)	0.427
Femoral neck (g/cm^2)	0.97 (±0.14)	0.98 (±0.11)	0.852
Mandibular ramus (g/cm^2)	0.50 (±0.29)	0.59 (±0.28)	0.359
Mandibular body (g/cm^2)	1.25 (±0.34)	1.46 (±0.34)	0.328

Table 3.1: Left-right differences between bite force, muscle activity and BMD for the <25yrs male and female cohorts.

Groups characteristics	Males (n=15) Mean (SD)	Females (n=15) Mean (SD)
Age (yrs)	20.93 (±1.71)	21.33 (±2.02)
Height (m)	1.79 (±0.07)**	1.66 (±0.06)**
Mass (Kg)	83.54 (±13.76)**	61.30 (±8.65)**

Table 3.2: Age, height and mass differences between<25yrs male and female cohorts. (** p< 0.01)

No significant difference existed between the average age of the male and female groups, but they did show a significant difference in average height ($p < 0.001$) and mass ($p<0.001$) (Table 3.2). Despite this difference, height and mass had no significant effect on the main outcome variables bite force, muscle activity and BMD ($p > 0.05$). However, mass did significantly correlate with BMD at the hip ($r=.603$, $p < 0.001$).

	Males	Females	*n*	MANOVA F	Observed Power	Univariate F	*P*
	Mean (SD)	Mean (SD)					
Main effects			30	0.363	0.125		ns
Max Bite Force (N)	295.3 (±142.6)	266.5 (±98.0)	30			0.416	ns
Max Masseter (%)	711.3 (±414.4)	616.1 (±256.4)	30			0.572	ns
Max Temporalis (%)	609.7 (±415.7)	563.1 (±252.5)	30			0.138	ns
BMD Ramus (g/cm^2)	0.65 (±0.27)	0.55 (±0.21)	30			1.247	ns
BMD mandible body (g/cm^2)	1.40 (±0.29)	1.366 (±0.40)	30			0.100	ns
EMG Masseter			30	0.820	0.224		ns
Max Masseter (%)	711.3 (±414.4)	616.1 (±256.4)	30			0.572	ns
75% Masseter (%)	577.2 (±287.8)	526.6 (±226.9)	30			0.286	ns
50% Masseter (%)	471.9 (±289.4)	372.4 (±116.3)	30			1.528	ns
25% Masseter (%)	285.9 (±158.8)	217.8 (±62.8)	30			2.382	ns
EMG Temporalis			30	0.068	0.061		ns
Max Temporalis (%)	609.7 (±415.7)	563.1 (±252.5)	30			0.138	ns
75% Temporalis (%)	494.9 (±323.9)	471.8 (±237.5)	30			0.049	ns
50% Temporalis (%)	368.3 (±229.2)	346.2 (±157.8)	30			0.095	ns
25% Temporalis (%)	231.5 (±147.5)	221.9 (±92.6)	30			0.045	ns
BMD effects			30	2.053	0.528		ns
BMD ramus (g/cm^2)	0.65 (±0.27)	0.55 (±0.21)	30			1.247	ns
BMD mandible (g/cm^2)	1.40 (±0.29)	1.36 (±0.40)	30			0.100	ns
BMD femoral neck (g/cm^2)	1.14 (±0.30)	0.98 (±0.10)	30			3.925	ns
BMD lumbar spine (g/cm^2)	0.90 (±0.23)	1.00 (±0.12)	30			2.235	ns

Table 3.3: MANOVA results for grouped dependent variables between the <25yrs male and female cohorts.

MANOVA results (Table 3.3) showed that males have higher bite force, muscle activity, femoral neck BMD and mandibular BMD but not lumbar spine BMD. However, these differences were not significant between males and females for any of the grouped variables: 'Bite force, muscle activity and mandibular BMD', 'EMG Masseter', 'EMG Temporalis' or 'BMD'.

Pearson's Correlation Coefficient					
	Max Bite Force (N)	Max Masseter (%)	Max Temporalis (%)	BMD ramus (g/cm^2)	BMD mandible (g/cm^2)
Males					
Max Bite Force (N)	-	-	-	-	-
Max Masseter (%)	0.59*	-	-	-	-
Max Temporalis (%)	0.03	0.50	-	-	-
BMD ramus (g/cm^2)	-0.16	0.02	0.33	-	-
BMD mandible (g/cm^2)	-0.10	0.22	0.25	0.32	-
Females					
Max Bite Force (N)	-	-	-	-	-
Max Masseter (%)	0.22	-	-	-	-
Max Temporalis (%)	0.47	0.56*	-	-	-
BMD ramus (g/cm^2)	-0.39	0.31	-0.04	-	-
BMD mandible (g/cm^2)	0.32	0.16	0.14	0.23	-

Table 3.4: Correlations between bite force, muscle activity and mandibular BMD for the <25yrs male and female cohorts.
(*$p<0.05$).

Table 3.4 shows significant correlations existed between maximum bite force and maximal masseter muscle activity ($r= .588$, $p<0.01$) in the male cohort and between the maximal masseter and maximal temporalis muscle activity ($r= .563$, $p<0.05$) in the female cohort.

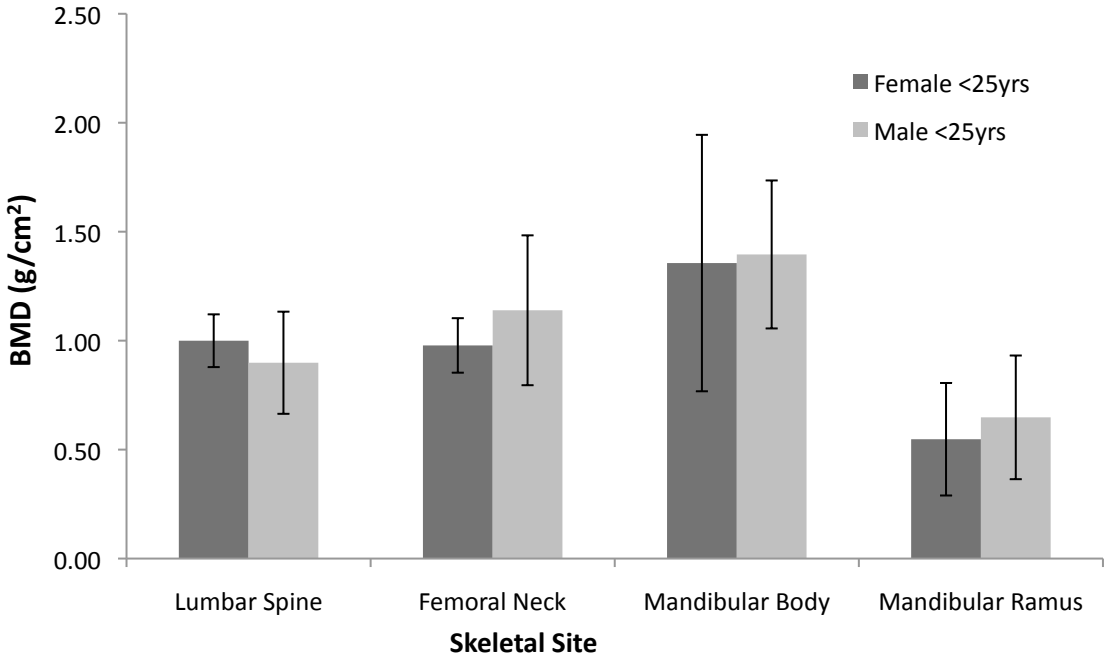


Figure 3.1: BMD values for lumbar spine, femoral neck mandibular body and ramus <25yrs male and female cohorts.

Pearson's Correlation Coefficient				
	BMD ramus (g/cm^2)	BMD mandible (g/cm^2)	BMD femoral neck (g/cm^2)	BMD lumbar spine (g/cm^2)
Males				
BMD ramus (g/cm^2)	-	-	-	-
BMD mandible (g/cm^2)	0.32	-	-	-
BMD femoral neck (g/cm^2)	0.00	0.24	-	-
BMD lumbar spine (g/cm^2)	0.39	0.16	-0.05	-
Females				
BMD ramus (g/cm^2)	-	-	-	-
BMD mandible (g/cm^2)	0.23	-	-	-
BMD femoral neck (g/cm^2)	0.00	-0.19	-	-
BMD lumbar spine (g/cm^2)	0.13	0.60*	0.44	-

Table 3.5: Correlations between BMD at skeletal sites for the <25yrs male and female cohorts.
(* p< 0.05, ** p< 0.01)

Figure 3.1 shows the mean and standard deviation BMD for each skeletal site, for both the male and female cohorts. The results show a 10.1% greater BMD at the lumbar spine in females compared to males, but a 14.2%, 2.8% and 15.5% greater BMD in males at the femoral neck, mandibular body and mandibular ramus respectively. Table 3.5 shows a significant correlation existed between the BMD of the mandibular body and the lumbar spine ($r=.596$, $p<0.05$) in females, but no significant correlations were found in the male cohort.

Discussion

This study examined the effect of sex on bite force, maximal muscle activity and bone mineral density variables. It specifically sought to explore the relationship between anatomical differences in males and females with bite force, muscle activity and BMD obtained from DXA. Furthermore, the study investigated the effect of sex on bite force, muscle activity and BMD as well as how the outcome variables correlated within each sample group.

Firstly, the results in Table 3.1 shows that the male and female sample groups were age-matched ($p>0.05$), but significant ($p<0.001$) differences in height and mass were present. These findings are concurrent with other studies (Barlett et al., 1991; Henry and Eastell, 2000; Riggs et al., 2004) and indicate a trend in greater skeletal size in males (Koc et al., 2010). Furthermore, analysis of the effect of height and mass on the main outcome variables showed no significant effect on maximum bite force, maximum muscle activity and BMD of the ramus and mandibular body. However, the

significant (r=.603, p<0.001) correlation between femoral neck BMD and mass suggests that body mass may have an effect on BMD at loaded skeletal sites, regardless of differences in sex. Similar studies reported the effect of skeletal size rather than mass on sex differences in BMD (Henry and Eastell, 2000).

Bone Mineral Density

Sex comparisons of the present study found no significant differences across any of the grouped bite force, EMG or BMD variables (p>0.05) (Table2). Despite the significant differences in height, there was no effect of sex on any of the bone mineral density outcome variables. Research suggests that sex differences in bone mineral density change or become non-significant when outcome variables are corrected for anatomical size (Henry and Eastell, 2000; Peacock et al., 2009), this correction was not used by Bakke et al. (1990). Both Henry and Eastell (2000) and Peacock et al. (2009) found men (aged 26yrs and 20-63yrs respectively) had greater bone size or volume, but similar or lower BMD than women at some skeletal sites, when corrected for the increase in bone size. This may be due to the planar nature of DXA, which most commonly measures the two dimensional area BMD rather than volumetric BMD, and may therefore under represent the density of the whole bone at skeletal sites (Riggs et al., 2004; Peacock et al., 2009). The present study modified the analysis technique for femoral neck BMD, so that the area of bone used for analysis was the same for every participant, regardless of bone size. This may have

corrected for anatomical differences between the two sample groups, which would explain the non-significant differences found between males and females, and is concurrent with previously mentioned literature. The absence of sex differences and correlation between skeletal sites in young adult cohorts may also be due to the variation in pre-peak bone mass values or continued development of the facial morphology in young adults (West and McNamara Jr et al., 1999). In the mandible, the eruption and/or removal of wisdom teeth may affect the load distribution and thereby the BMD.

The female sample group exhibited a significant correlation between mandibular BMD and the lumbar spine, which is similar to Drage et al. (2007) who also found correlations between facial BMD and the lumbar spine. However, Drage et al. (2007) found correlations between BMD at the ramus and the lumbar spine, in a cohort of edentulous subjects in mid to late adulthood. In relation to the present study group, all participants were free from musculoskeletal disease and fully dentate. Studies conducted using older participants may help to indicate a strong correlation between BMD at facial and loaded skeletal sites, but these findings may be age related and therefore are not be applicable to the current sample group of young adults that have not yet reached peak bone mass. The correlation between mandibular and lumbar spine BMD in females in the present study may indicate the semi-loaded nature of the bone tissue in both the mandibular body and the lumbar spine. The lumbar spine is loaded by the upper body but does not often experience loading from ground reaction forces. The mandibular body is commonly loaded through the dentition during mastication, but rarely experiences high loads as

chewing is most commonly performed at a sub-maximal level. In young active adults, with sound muscle activity, this may indicate the homeostasis of semi-loaded bone tissue. In terms of facial injury in sport, lower BMD values in the young female population may indicate a susceptibility to injury in comparison to males, due to reduced strength and integrity of the facial bones.

Bite Force and Muscle activity

The present study found no effect of sex on any of the bite force and muscle activity variables in a cohort of young adults aged 18-25yrs. These findings comply with Lepley et al. (2011) who reported no sex difference in a similar cohort of young adults aged 22-32yrs. Bakke et al. (1990) reported significant correlations between height and bite force in males and females under the age of 25 years, as well as significant differences in bite force between sexs, in a cohort of 63 females and 59 males. This study utilised far greater sample sizes than the present study, which may explain the significant differences between sexs compared to the non-significant findings of the present study. Existing bite force and muscle activity literature has reported non-significant results in relation to sex; Lindauer et al. (1993) found no significant differences in EMG-force slopes at the masseter or anterior temporalis between sexs and Fogle and Glaros (1995) reported no significant differences in incisal bite force and muscle activity at the masseter and temporalis between sexs. Shinogaya et al. (2001) found maximal clenching forces were higher in Japanese males than females. However, they also found that the number of occlusal contacts

was higher in males but the average pressure (force divided by occlusal contact area) between the two sexs was not significant. This would suggest that maximal bite force is affected by occlusal contacts and that their perceived sex difference in maximal bite force is offset by the occlusal contact area. Therefore, it is probable that craniofacial morphology, in particular the biomechanics of the mandible, and occlusion may have a greater effect on force production than sex (Bakke, 2006). If this is so, the size of the sample groups used in the present study may be insufficient for detecting the effect of sex over the effect of craniofacial morphology or occlusal variability. The final chapter of the present study will address the differences in craniofacial dimensions and the effect they have on bite force, jaw elevator muscle activity and mandibular BMD. The previously mentioned studies that found no significant sex differences comprised of small sample groups: Lindauer et al. (1993) 8 males, 8 females, Fogle and Glaros (1995) 12 males and 12 females, and Shinogaya et al. (2001) 12 males and 12 females. These sample sizes are similar to the present research, which used a sample group of 15 males and 15 females, and is concurrent with Lepley et al. (2011) who also found no sex difference in bite force. Conversely, studies that have reported significant differences between males and females, of the same age and ethnicity, have utilised larger sample groups for their comparisons (Ferrario er at., 1993; Raadsheer et al., 2004; Palinkas et al., 2010). Palinkas et al. (2010) reported sex as a significant factor associated with bite force and muscle thickness, but not muscle activity, in the masseter and anterior temporalis, at all age levels from 7-80yrs (20 males and 20 females). Raadsheer et al. (2004) showed significant differences across bite force, moments and muscle thickness in the face,

upper limb and lower limb between sexs (57 males and 64 females, mean age 23yrs). Similarly, Ferrario et al. (1993) found significantly higher muscle activity in the masseter in males during maximal clenching (49 males and 43 females). Furthermore, Van Der Bilt et al. (2008) (13 males and 68 females) found sex had a significant effect on bilateral bite force and Visser et al. (1994) (40 males and 20 females) found significant differences in submaximal muscle activity at the masseter and temporalis. However, the uneven sample groups in those studies may have affected the significance of the findings. It is unclear whether the present findings were hindered by sample size, the groups were of equal size and were normally distributed but may have been too small to identify a significant difference in bite force between sexs.

It is difficult to fully compare the results from facial EMG studies as authors tend to report muscle activity in differing sample sizes and methodologies, which may also take into account various other craniofacial variables, such as long term migraines and osteoporosis of the jaw (Burnett et al., 2000; Suvinen and Kemppainen, 2007; Siéssere et al., 2009). Ferrario et al. (2004) conducted a facial EMG study using healthy young adults, they reported no sex differences, however the sample groups were minimal and uneven (2 females, 8 males). Ferrario et al. (2000) conducted a facial EMG study that measured muscle activity from the masseter and anterior temporalis, on 15 males and 15 females aged 18-19yrs, the study found no sex difference and subsequently pooled the data. Again, sample group sizes (15 males and 15 females) may have been too small to detect the difference in muscle activity between sexs in the present study. As no significant differences between sex were

identified in any of the outcome variables, the male and female data can be utilised both separately and pooled for further analysis, similar to Ferrario et al., (2000).

Correlation results showed significantly strong, positive relationships between maximum bite force and maximal masseter muscle activity in males, as well as a significantly strong, positive correlation between maximal masseter and maximal temporalis muscle activity ($r = .563$, $p<0.01$) in the female cohort (Table 3.4). These findings are concurrent with Ferrario et al. (2004) who reported a linear relationship between bite force and facial muscle activity in males and females, in the pooled EMG data from the masseter and anterior temporalis. Additionally, Lindauer et al. (1993) found a significant increase in muscle activity (at the masseter and anterior temporalis), with an increase in bite force at a jaw opening width of 7mm at the molars. The size of the transducer used in the present study was 8.8mm high, which results in a greater but similar molar opening height to that reported by Lindauer et al. (1993). Molar jaw opening heights less than 9mm may reflect a biomechanical optimum for utilising the muscle-force relationships, which could fit with existing literature on incisal opening heights (Paphangkorakit and Osborn, 1997). The correlations between bite force and masseter muscle activity as well as masseter and temporalis muscle activity in the present study may simply reflect the functional, linear relationship between muscle activity and bite force. In young, healthy muscle, an increase in force is likely to correspond to an increase in activity of the contracting muscles. Therefore, these findings show the under 25yrs males and females exhibit a healthy, functional relationship between muscle activity and bite force.

Conclusion

This study found that sex had no significant effect on bite force, jaw elevator muscle activity and mandibular BMD at any of the measured skeletal sites, in a <25yrs cohort. These findings are concurrent with some previous literature. Bite force and muscle activity correlated within sample groups, with different correlations present in males than females. Sample size may have negatively influenced the present findings, which may have prevented the discovery of significant differences between sexs. The correlations between bite force and muscle activity, indicate a functional, linear relationship between bite force and muscle activity in a young adult population, which may be used as a control group to compare to other sample groups.

The Subsequent Chapter

The following chapter will focus on the effect of age on bite force, muscle activity and BMD, specifically examining the differences between males and females over the age of 50 years and how they compare to the under 25 years age group. Age related changes in BMD, muscle strength and dentition have been reported in literature (Broadbent et al., 2006; Goodpaster et al., 2006; Looker et al., 2009), which may result in differences in bite force and facial muscle activity, thus having an effect on mandibular BMD. A reduction in BMD of the facial bones has been

reported during ageing, specifically within a female cohort (Devlin and Horner, 2007).

Chapter 4: The Effect of Age on Bite force, Muscle activity and BMD.

Introduction

The onset of ageing causes physical and detrimental changes to the human body, which includes a reduction in muscle strength and mass (Gallagher et al., 1997; Goodpaster et al., 2006), decrease in fat free mass to height ratio (Barlett et al., 1991), and a decrease in axial skeletal height (Perissinotto et al., 2002). Changes and adaptations of the human skeleton in size, shape and biomechanical properties occur throughout childhood and adolescence into adulthood, which can influence injury or fracture (Skrzat et al., 2004; Looker et al., 2009; Verschueren et al., 2013). During ageing, decreases in trabecular volumetric bone mineral density (vBMD) occur across skeletal sites (Riggs et al., 2004) with further decreases in cortical vBMD up to 25% in old age (Looker et al., 2009), thus increasing the risk of fracture during ageing (Seeman 2008b).

Bone mineral density (BMD) is a key indicator of bone strength and can indicate the onset of metabolic bone diseases such as osteopenia and osteoporosis. Key skeletal sites for determining bone loss with age include the lumbar spine and femoral neck, these anatomical sites are examined throughout this research study. During ageing, the human skeleton resorbs a greater amount of bone than it regenerates, which results in a natural decrease in bone density (Frost, 2002). This produces a net loss in bone and therefore a reduction in material properties such as density and

toughness. In a cadaveric study examining mid-diaphyseal femur samples, Zioupos (2001) reported that the process of ageing causes architectural changes, specifically an increase in cortical porosity, which reduces the material stiffness and strength of bone. These changes in biomechanical properties increase the risk of fracture in ageing bone tissue, as an increase in porosity inevitably reduces the density of the bone. Furthermore, Parfitt et al. (1983) reported a steady decrease in trabecular bone volume in men and women aged 50-80yrs. However, the study highlighted the differences between trabecular plate density, which irreversibly decreases with age and trabecular bone thinning, which can be maintained during ageing. Parfitt et al. (1983) also highlighted that the key to biomechanically sound trabecular bone lies in the connection of the struts to other bone tissue, rather than their thickness or volume which is important in the prevention of bone loss.

Looker et al. (2009) examined bone density of males and females aged 50-80+yrs. The study reported higher mean BMD values for the pelvis at the age of 50 compared to 80+yrs in females (1.25g/cm^2 for females aged 50yrs and 1.0g/cm^2 for females aged 80+yrs). Similarly, the male cohort exhibited higher mean BMD values at 50yrs than 80+yrs (1.3g/cm^2 at 50yrs and 1.1g/cm^2 at 80+yrs). Additionally, the study reported mean BMD at the lumbar spine of 1.10g/cm^2 at 50yrs and 1.0g/cm^2 at 80+yrs in females. These values highlight a 25% reduction in BMD at the pelvis in females and a 20% reduction in males, as well as a 10% reduction in BMD at the lumbar spine in females, between the age of 50yrs and 80+yrs. Furthermore, lumbar spine BMD increased about 10% with age in males, which could be attributed to the confounding changes observed in the spine, particularly vascular calcification of

vertebral mineral density, due to age (Looker et al., 2009). It is possible to maintain or slow the natural loss of BMD at these sites through exercise and loading (Marques et al., 2012; Allison et al., 2013). Allison et al. (2013) conducted a 12 month high impact unilateral exercise intervention on men aged 65-80yrs, which involved performing multidirectional hops 7 days a week. The exercise leg increased in BMD at the femoral neck from 0.948 ±0.02g/cm^2 to 0.954 ±0.02g/cm^2, at the trochanter from 0.920 ±0.02g/cm^2 to 0.923 ±0.02g/cm^2, and at the total hip from 1.027 ±0.02^g/cm2 to 1.030 ±0.02g/cm^2. Conversely, the control leg decreased in BMD at the femoral neck from 0.954 ±0.02g/cm^2 to 0.945 ±0.02g/cm^2, at the total hip from 1.029 ±0.02^g/cm2 to 1.027 ±0.02g/cm^2, but an increase was observed at the trochanter from 0.919 ±0.02g/cm^2 to 0.923 ±0.02g/cm^2. Moreover, poor health in men and women, or rapid BMD loss in postmenopausal women, can have greater negative effects than the gradual BMD loss during ageing, but may be counteracted by exercise interventions, or drug therapy (McClung et al., 1998; Black et al., 2000; Winters-Stone et al., 2012; Verschueren et al., 2013).

Ageing and the Craniofacial skeleton

Age causes structural bone change and geometrical changes to occur to the craniofacial skeleton in humans (Gray, 2010), which manifests as a slow decrease in parietal bone remodelling (Torres-Lagares et al., 2010), decreases in facial height, and increases in dental attrition (Albert et al., 2007). Furthermore, during ageing the material properties throughout the dentate mandible exhibit extreme regional

variations, with significant ($p<0.001$) differences in cortical bone thickness and stiffness (Schwartz-Dabney and Dechow, 2003). These regional variations alongside that of bone density, have a vital impact on mandibular mechanics and may be crucial to understanding the differences in fracture mechanics of the mandible at different impact sites. In relation to edentulous cohorts Drage et al. (2007) reported that BMD of the ramus correlated with that of the femoral neck and lumbar spine in a small cohort (9m, 9f) of participants aged 67±12.6yrs. Horner et al. (1996) measured BMD of the mandible in a cohort of healthy, edentulous females aged 44-79yrs (mean 65yrs). The study found significant correlations between the mandibular ramus and mandibular body ($p<0.001$), as well as the mandibular body and the mandibular symphysis ($p=0.008$). Similarly, the study found significant correlations between mandibular body and lumbar spine ($r=0.49$, $p=0.001$) and femoral neck ($r=0.45$, $p=0.004$), as well as mandibular ramus and lumbar spine ($r=0.52$, $p=0.001$) and femoral neck ($r=0.38$, $p=0.016$). Furthermore, in a large cohort of Iranian men and women aged 55±10.8yrs categorised as 'normal', 'osteopenia' and 'osteoporosis' by BMD values, the regions of the maxilla and mandible significantly correlated with hip and lumbar spine measurements using DXA (Esfahanizadeh et al., 2013). Lindh et al. (2004) measured a small cohort (10m, 8f) of mid to old aged (51-79yrs) edentulous participants, the results showed maxillary BMD varied significantly between individuals, but also significantly correlated with BMD of the lumbar spine. In addition, Geraets et al. (2014) reported that BMD of the upper and lower jaw could be analysed from radiographs and used to predict the BMD of the hip and lumbar spine from DXA measurements. The majority of previous studies focus on

postmenopausal females and in most cases the subjects are edentulous/partially dentate, which is an important factor because tooth loss has been found to significantly ($p=0.017$) affect osteoporotic status (Darcey et al., 2013).

Muscular changes with Ageing

The properties of muscles undergo changes with ageing; Merletti et al. (2002) found a significant difference ($p<0.01$) in EMG mean frequency in 20-30 year olds compared to over 60yrs during submaximal biceps contraction, these findings were attributable to differences in fibre type distribution and a decrease in motor unit firing rates with age. These changes are likely to decrease maximal contraction capabilities and increase muscular fatigue (Merletti et al., 2002; Goodpaster et al., 2006). Similarly, within the craniofacial muscles activity during maximal clenching, clinical movements and rest have been found to be significantly ($p<0.05$) different from childhood through adolescence, adulthood and into old age. CecÍLio et al. (2010) reported a significant ($p<0.05$) decrease of 3-5% masseter and 15-16% temporalis muscle activity from young adulthood (aged 21-40yrs) to old age (aged 61-80yrs). Thus, with a reduction in muscle activity, it is pertinent to suggest that this would detrimentally effect the bone mineral density through lower muscle strains. This detrimental effect has been seen within muscle sarcopenia in old age, which has been shown to have a negative effect on the density of long bones. Age related osteoporosis has also been found to cause increases in muscle activity during rest or submaximal movements but lower activity in maximal clenching (Siéssere et al.,

2009), indicating a dependency on muscle activity for mandibular stabilisation but a reduction in maximal muscle contraction. Furthermore, Galo et al. (2007) observed a reduction in normalised muscle activity in the elderly when chewing hard foods but no difference in consistency at lower levels, which suggests the elderly lose maximal capacity but remain efficient at low bite force levels. This may be explained by a decrease in muscle recruitment with age (Fogle and Glaros, 1995) or may be linked to the strength and condition of participants' teeth, which can heavily influence their capability to bite through dense food (Peyron et al., 2004). Thus, the use of a submaximal (20N) bite force for the EMG normalisation technique utilised within the present study as described in Chapter 2 Section 2.4, will benefit older participants who are less capable of performing maximal voluntary bite forces. Bite force capabilities have been reported to decrease with increasing age regardless of muscle thickness; for example Palinkas et al. (2010) reported males aged 13-20yrs produced a mean bite force of 405±30N whereas males aged 41-60yrs produced a mean bite force of 323±34N. Similarly, females aged 13-20yrs produced a mean bite force of 280±31N compared to females aged 61-80yrs who produced a mean bite force of 162±46N. It has also been found that the number of occluding teeth has a greater effect on bite force than the number of teeth in the mouth (Bakke et al., 1990), but tooth loss has been significantly ($p=0.017$) linked to a reduction in bone mineral density (Darcey et al., 2013), which may in turn affect bite force. A reduction in BMD in the mandible could increase fracture risk at that site, particularly in adults who participate in sport and recreational activity.

As previously mentioned, loading and physical activity, maintain or increase BMD, however, the number of adults participating in sport and recreation in the UK between the ages of 55-84yrs is considerably lower than the number of younger participants. Of the Caucasian respondents, 16% of 55-64 year olds, 12% of 65-74 year olds and 6% of 75-84 year olds took part in 3x 30 minutes of sport and active recreation a week, as reported by Long et al. (2009) in the Active Peoples Survey. Men and women who participate in sport for recreation and/or competition will benefit by maintaining BMD longer at loaded skeletal sites and reducing the natural effect of ageing on skeletal muscle, as reported by Wilks et al. (2009) in a cohort of 300 master athletes (aged 35-94yrs). However, reduced facial BMD may be caused by loss of dentition in an individual and thereby increase the risk of facial injury, whilst the lower limb BMD is maintained through repeated loading. It is therefore important to consider the additional risk that Master athletes and those who participate in recreational sport and activity are at, due to the age related reduction in skeletal integrity.

Aims and Objectives

The aim of the present study was to investigate the effect of age on mandibular bone mineral density, muscle activity and bite force capabilities of a cohort of Caucasian men and women, comparing them to the data obtained in the young adult cohort in Chapter 3. The objectives of the study were to;

(i) Identify how the bite force, muscle activity and BMD at all skeletal sites relate to anatomical differences in sample groups.

(ii) Identify whether age affected bite force, maximal muscle activity and facial BMD.

(iii) Investigate how bite force, muscle activity and BMD at all skeletal sites correlated within each sample group.

Materials and Methods

Prior to commencement of the study ethical approval was obtained and granted from the Department of Exercise and Sport Science Research Ethics Committee, Manchester Metropolitan University. The methodological procedures are described in Chapter 2 Section '2.6 Materials and Methods', the only differences are the details concerning the sample groups, which are included below.

Participants

Exclusion criteria for the study was a previous history of facial fracture or facial surgery, current or recent orthodontic treatment/surgery, dental treatment within 6 months that consisted of more than a routine check-up (particularly including X-ray or CT scanning). Additionally, long term parafunctional habits such as bruxism, temporomandibular dysfunction or masticatory pain, or musculoskeletal conditions

that were known to affect bone metabolism. A total of 60 Caucasian participants were recruited which consisted of an overall experimental group of 15 young males and 15 young females aged 18-25yrs (mean age 21.1±1.9yrs) and 15 males and 15 females aged 50+yrs (mean age 63.0±7.1yrs). All participants were informed of the study procedures and gave written informed consent prior to taking part in the study.

Data Analysis

T-tests were used to calculate the difference in each outcome variable between males and females in the over 50yrs cohort (Tables 4.1). A one-way MANOVA was performed on the bite force, muscle activity and bone mineral density data (SPSS statistical analysis software 19). Each MANOVA measured the effect of age on four grouped dependent variables, as shown in Table 4.3. The significance level was set to $p< 0.05$ and the univariate significance level was set to $p< 0.0125$ using the Bonferroni correction for four dependent variables and $p< 0.01$ for five dependent variables (Field, 2009). Levene's test for homogeneity (across all conditions) indicated equal variances ($p>0.05$) for all independent comparisons, therefore the assumption of homogeneity was met. Skewness and Kurtosis analysis indicated that the z-values for all variables were within the ±1.96 range, therefore they did not differ significantly from normality.

Pearson's correlation coefficients were calculated between the main dependent variables (Maximum bite force, Maximum Masseter muscle activity. Maximum anterior Temporalis muscle activity, BMD at the Ramus and Mandibular body) as well as the four BMD measurement sites (Mandibular Ramus, Mandibular body, Femoral neck and Lumbar spine).

Results

The results in Table 4.1 shows the sex difference in the over 50yrs cohort, which highlights significantly higher bite force ($p<0.01$) and masseter muscle activity ($p<0.05$) in males than females. There were no significant ($p>0.05$) differences in BMD at any skeletal site between sex in the over 50yrs group. Due to the significant difference in bite force and masseter muscle activity in the >50yrs cohort, the under 25yrs and over 50yrs groups were divided into males and females to investigate the effect of age on the outcome variables. When pooled, the group characteristics showed non-significant differences in height and mass between the age groups, and a significant ($p<0.05$) difference in BMI (Table 4.2).

	Males >50yrs Mean (SD)	Females >50yrs Mean (SD)	*n* 30	Univariate F	*P*
Max Bite Force (N)	258.7 (±93.5)	162.0 (±76.1)		9.65	**
Max Masseter (%)	544.7 (±442.3)	273.6 (±104.6)		5.34	*
Max Temporalis (%)	432.9 (±229.8)	323.4 (±187.1)		2.04	ns
BMD ramus (g/cm^2)	0.72 (±0.15)	0.58 (±0.23)		4.13	ns
BMD mandible (g/cm^2)	1.165 (±0.30)	0.99 (±0.41)		1.48	ns
BMD Femoral neck (g/cm^2)	0.89 (±0.22)	0.81 (±0.16)		1.13	ns
BMD Lumbar spine (g/cm^2)	0.87 (±0.35)	0.91 (±0.19)		0.14	ns

Table 4.1: Differences in bite force, muscle activity and BMD between >50yrs male and female cohorts.

(***** p< 0.05, ****** p< 0.01).

	Under 25yrs	*n*	Over 50yrs	*n*
Group Characteristics		30		30
Age (yrs)	21.13 (±1.85) *		62.96 (±7.12) *	
Height (m)	1.72 (±0.09)		1.71 (±0.11)	
Mass (Kg)	72.42 (±15.98)		79.24 (±20.62)	
BMI	24.17 (±3.99) *		26.79 (±5.89) *	

Table 4.2: Age, height, mass and BMI differences between both the pooled <25yrs and >50yrs cohorts.
(*p<0.05)

Investigation of the effect of body size on BMD showed mass significantly (r=.387, p<0.05) correlated with femoral neck BMD in the over 50s group, which is a similar finding to previously reported (r=.603, p< 0.01) correlations in Chapter 3, within the under 25yrs cohort.

	Under 25yrs Female	Over 50yrs Female	*n*	MANOVA F	Observed Power	Univariate F	*P*
	Mean (SD)	Mean (SD)					
Main Effects			30	12.61	1.0		***
Max Bite Force (N)	266.46 (±97.96)	162.02 (±76.06)				10.64	†
Max Masseter (%)	616.05 (±256.39)	273.57 (±104.55)				22.95	†
Max Temporalis (%)	563.14 (±252.53)	323.43 (±187.11)				8.72	†
BMD ramus (g/cm^2)	0.55 (±0.21)	0.58 (±0.23)				0.156	ns
BMD mandible (g/cm^2)	1.36 (±0.59)	0.99 (±0.41)				11.68	†
BMD Effects			30	4.78	0.91		**
Ramus (g/cm^2)	0.55 (±0.21)	0.58 (±0.23)				0.156	ns
Mandible (g/cm^2)	1.38 (±0.59)	0.99 (±0.41)				11.68	†
Femoral neck (g/cm^2)	0.98 (±0.10)	0.81 (±0.17)				10.63	†
Lumbar spine (g/cm^2)	1.00 (±0.12)	0.91 (±0.19)				2.60	ns

Table 4.3: MANOVA results for grouped dependent variables for the <25yrs and >50yrs females.

(* p< 0.05 **** p< 0.01 *****p<0.001 **†**Significant using Bonferroni correction for MANOVA with 4 dependent variables (p<0.0125) or with 5 dependent variables (p<0.01)).

Table 4.3 shows that there was a significant (p≤0.0001) effect of age on the grouped dependent variables (maximum bite force, maximum muscle activity and BMD at the ramus and mandibular body) in the female cohorts. Additionally, there was a significant (p<0.01) effect of age on the grouped BMD variables (ramus, mandibular body, femoral neck and lumbar spine) in the female cohorts (Table 4.3). When the Bonferroni correction was applied, maximum bite force (p=0.003), maximum masseter activity (p≤0.0001), maximum temporalis activity (0.006), femoral neck BMD (p=0.003) and mandibular BMD (p=0.002) were significantly affected by age (p<0.0125) in the female cohorts (Table 4.3).

	Under 25yrs male	Over 50yrs male	*n*	MANOVA F	Observed Power	Univariate F	*P*
	Mean (SD)	Mean (SD)					
Main Effects			30	1.742	0.50		ns
Max Bite Force (N)	295.26 (±142.57)	258.71 (±93.55)				0.69	ns
Max Masseter (%)	711.25 (±414.4)	544.69 (±442.27)				1.13	ns
Max Temporalis (%)	609.72 (±415.74)	432.85 (±229.84)				2.08	ns
BMD ramus (g/cm^2)	0.65 (±0.26)	0.72 (±.15)				0.87	ns
BMD mandible (g/cm^2)	1.40 (±0.29)	1.16 (±.30)				4.81	*
BMD Effects			30	2.59	0.64		ns
Ramus (g/cm^2)	0.65 (±0.26)	0.72 (±0.15)				0.87	ns
Mandible (g/cm^2)	1.40 (±0.29)	1.16 (±0.30)				4.81	*
Femoral neck (g/cm^2)	1.14 (±0.30)	0.89 (±0.22)				7.05	†
Lumbar spine (g/cm^2)	0.90 (±0.23)	0.87 (±0.35)				0.08	ns

Table 4. 4: MANOVA results for grouped dependent variables for the <25yrs and >50yrs males.

(* p< 0.05 ** p< 0.01 ***p<0.001 †Significant using Bonferroni correction for MANOVA with 4 dependent variables (p<0.0125) or with 5 dependent variables (p<0.01)).

Table 4.4 shows that there was no significant (p>0.05) effect of age on the grouped dependent variables (maximum bite force, maximum muscle activity and BMD at the ramus and mandibular body) in the male cohorts. Additionally, there was no significant (p>0.05) effect of age on the grouped BMD variables (ramus, mandibular body, femoral neck and lumbar spine) in the male cohorts (Table 4.4). When the Bonferroni correction was applied, only femoral neck BMD (p=0.012) was significantly affected by age, in the male cohorts. Mandibular BMD (p=0.037) was significantly affected when analysed at a univariate level (Table 4.4).

Pearson's Correlation Coefficient					
<25yrs Females	Max Bite Force (N)	Max Masseter (%)	Max Temporalis (%)	BMD ramus (g/cm^2)	BMD mandible (g/cm^2)
Max Bite Force (N)	-	-	-	-	-
Max Masseter (%)	0.22	-	-	-	-
Max Temporalis (%)	0.47	0.56*	-	-	-
BMD ramus (g/cm^2)	-0.39	0.31	-0.04	-	-
BMD mandible (g/cm^2)	0.32	0.16	0.136	0.232	-
>50yrs Females					
Max Bite Force (N)	-	-	-	-	-
Max Masseter (%)	0.46	-	-	-	-
Max Temporalis (%)	-0.07	0.54*	-	-	-
BMD ramus (g/cm2)	0.32	0.14	0.06	-	-
BMD mandible (g/cm2)	0.74**	0.50	0.18	0.76**	-

Table 4.5: Correlation between bite force, muscle activity and BMD for the <25yrs and >50yrs female cohorts.

(*p<0.05, **p<0.01)

Table 4.5 shows a significant correlations (r=.563, p<0.05) between masseter muscle activity and temporalis muscle activity in the female <25yrs group. In the female >50yrs group, significant correlations between bite force and mandibular body BMD (r=.738, p<0.01), Masseter muscle activity and temporalis muscle activity (r=.536, p<0.05) and between ramus BMD and mandibular body BMD (r=.756, p<0.01) were identified (Table 4.5).

Pearson's Correlation Coefficient					
<25yrs Males	Max Bite Force (N)	Max Masseter (%)	Max Temporalis (%)	BMD ramus (g/cm^2)	BMD mandible (g/cm^2)
Max Bite Force (N)	-	-	-	-	-
Max Masseter (%)	0.59*	-	-	-	-
Max Temporalis (%)	0.03	0.50	-	-	-
BMD ramus (g/cm^2)	-0.16	0.02	0.33	-	-
BMD mandible (g/cm^2)	-0.10	0.22	0.25	0.32	-
>50yrs Males					
Max Bite Force (N)	-	-	-	-	-
Max Masseter (%)	-0.04	-	-	-	-
Max Temporalis (%)	0.18	0.51*	-	-	-
BMD ramus (g/cm^2)	0.53**	-0.35	-0.17	-	-
BMD mandible (g/cm^2)	0.09	-0.37	-0.01	0.12	-

Table 4.6: Correlation between bite force, muscle activity and BMD for the <25yrs and >50yrs male cohorts.

(*$p<0.05$, **$p<0.01$)

Table 4.6 shows significant correlations ($r=.588$, $p<0.05$) between bite force and masseter muscle activity in the male <25yrs group only. In the male >50yrs group, significant correlations between bite force and ramus BMD ($r=.532$, $p<0.01$) and between masseter muscle activity and temporalis muscle activity ($r=.509$, $p.<0.05$) were identified (Table 4.6).

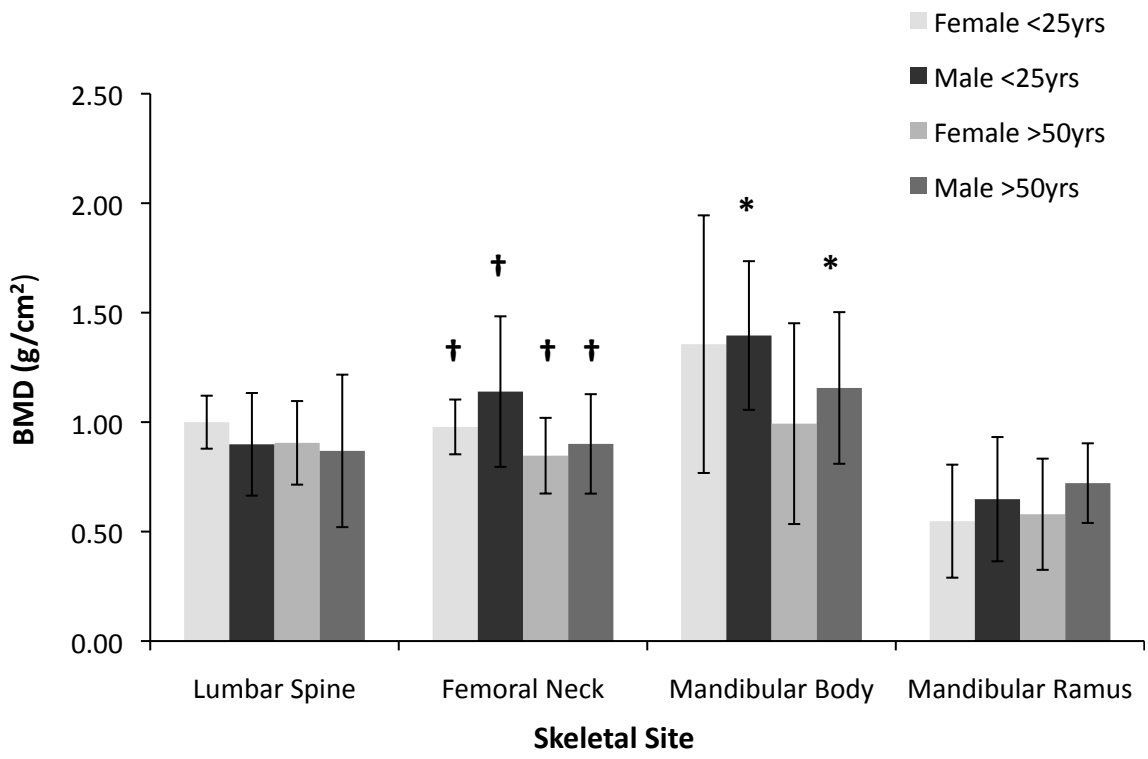


Figure 4.1: BMD values for lumbar spine, femoral neck, mandibular body and ramus for the <25yrs and >50yrs male and female cohorts.

(*p<0.05, †Significant (p<0.0125) using Bonferroni correction for MANOVA with 4 dependent variables.)

Figure 4.1 shows the BMD values for each sex and age group. Males had consistently higher BMD at the femoral neck, mandibular body and mandibular ramus compared to females, regardless of age. These were statistically different ($p<0.0125$) at the femoral neck in the female and male cohorts and at the mandibular body ($p<0.05$) in the male cohorts. The <25yrs had consistently higher BMD at the lumbar spine, femoral neck and mandibular body than the >50yrs regardless of sex, but exhibited lower BMD at the mandibular ramus. This may be due to continued facial bone growth or wisdom tooth eruption during early adulthood (West and McNamara Jr, 1999).

Pearson's Correlation Coefficient				
Females <25yrs	BMD ramus (g/cm^2)	BMD mandible (g/cm^2)	BMD femoral neck (g/cm^2)	BMD lumbar spine (g/cm^2)
BMD ramus (g/cm^2)	-	-	-	-
BMD mandible (g/cm^2)	0.23	-	-	-
BMD femoral neck (g/cm^2)	0.00	-0.19	-	-
BMD lumbar spine (g/cm^2)	0.13	0.60*	0.44	-
Females >50yrs				
BMD ramus (g/cm^2)	-	-	-	-
BMD mandible (g/cm^2)	0.76**	-	-	-
BMD femoral neck (g/cm^2)	0.05	-0.17	-	-
BMD lumbar spine (g/cm^2)	0.18	0.34	0.07	-

Table 4.7: Correlation between BMD at skeletal sites for the <25yrs and >50yrs female cohorts.

(* p< 0.05, ** p< 0.01)

Pearson's Correlation Coefficient				
Males <25yrs	BMD ramus (g/cm^2)	BMD mandible (g/cm^2)	BMD femoral neck (g/cm^2)	BMD lumbar spine (g/cm^2)
BMD ramus (g/cm^2)	-	-	-	-
BMD mandible (g/cm^2)	0.32	-	-	-
BMD femoral neck (g/cm^2)	0.00	0.24	-	-
BMD lumbar spine (g/cm^2)	0.39	0.16	-0.05	-
Male >50yrs				
BMD ramus (g/cm^2)	-	-	-	-
BMD mandible (g/cm^2)	0.12	-	-	-
BMD femoral neck (g/cm^2)	-0.26	0.15	-	-
BMD lumbar spine (g/cm^2)	0.38	0.67**	0.10	-

Table 4.8: Correlation between BMD at skeletal sites for the <25yrs and >50yrs male cohorts.

(* p< 0.05, ** p< 0.01)

Table 4.7 shows that mandibular BMD significantly correlated with lumbar spine ($r=.596$, $p<0.05$) in the female <25yrs cohort and mandibular BMD significantly correlated with ramus BMD ($r=.761$, $p<0.01$) in the female >50yrs cohort. Table 4.8 shows that none of the BMD skeletal sites significantly correlated ($p>0.05$) in the male <25yrs, whereas the mandibular BMD significantly correlated with lumbar spine ($r=.673$, $p<0.01$) in the male >50yrs cohort.

Discussion

The present study investigated the effect of age on bite force, jaw elevator muscle activity and mandibular bone mineral density, in a cohort of white Caucasian men and women. The study aimed to identify whether anatomical differences in sample groups affected the outcome variables, identify whether age affected the bite force, maximal muscle activity and mandibular BMD within male and female cohorts, and finally examine how the outcome variables correlated within each sample group. The sample groups had similar group means for height and mass but were significantly different for BMI. The <25yrs sample group exhibited a normal BMI score, whereas the >50yrs group were categorised as overweight. Nevertheless, it is considered that healthy men and women aged over 50 years will present a BMI score about 20 (higher than the normal range) and this should be considered normal (Perissinotto et al., 2002). Height and mass are likely to affect BMD at sites such as the femoral neck, due to increased loading and size related increases in bone mass (Riggs et al., 2004),

this is reflected in the significant correlations between mass and femoral neck BMD in the present study.

Bite Force and Muscle Activity

The main findings showed age had a significant effect on the main outcome variables (maximum bite force, maximum muscle activity and BMD at both sites on the mandible) in the female cohort, which indicate an overall ageing effect on the stomatognathic system. However, there was no significant effect of age on bite force, muscle activity or BMD in the male cohort. At a univariate level, bite force, masseter muscle activity, temporalis muscle activity and mandibular BMD were all significantly different between <25yrs and >50yrs females. In the male cohort only the mandibular BMD was significantly different between <25yrs and >50yrs. These findings indicate a reduction in bite force with increasing age in females, which is concurrent with studies that describe a reduction in incisal biting force (Fogle and Glaros, 1995) and molar biting force (Palinkas et al., 2010) in adults, as age increases. The present study also found a significant reduction (104.5N) in mean maximal molar bite force in the >50s females but the reduction in >50yrs males (36.6N) was not significantly different from their younger counterparts. These results are similar to the findings of Palinkas et al. (2010) who reported an 82N maximal bite force reduction in males (from 405±3N to 323±3N) and 118N maximal bite force reduction in females (from 280±3N to 162±5N), between under 13-20yrs and 41- 60yrs. There are a number of attributable factors that may cause this decline; the number of

occluding teeth, reduced muscular capabilities or a reduced stability in the jaw as a combination of poor dentition, reduced BMD integrity and reduced muscle pull, as well as the use of dentures in an older population (Caloss et al., 2010). The over 50yrs group in the present study consisted of mainly dentate participants with some false dentition (63%) of those 11% wore dentures and of the total number 37% were fully dentate. However, there was no statistical ($p>0.05$) difference between dental condition between males and females in the over 50s group, to explain the small non-significant decrease in male bite force compared to the large significant decrease in female bite force. Furthermore, there was no apparent or statistical ($p>0.05$) relationship between false dentition/dentures and low bite force. The large decrease in females compared to males in the present study may reflect the chronic effects of menopause alongside the natural process of ageing, on the muscle-bone relationship, which results in reduced force due to lower muscle strains and bone tissue integrity. Galo et al. (2007) reported a lower mean muscle activity in the elderly compared to the young, when chewing hard foods but no difference in consistency at lower levels, which suggests the elderly remain efficient at low bite force levels. The EMG normalisation technique used in this study was therefore appropriate for use with the >50yrs cohort to compare maximal bite force capability to bite forces that were comfortably within their ability. Similar findings have been reported by Peyron et al. (2004), who observed that in comparison to young adults, older participants required a greater number of chewing cycles to break down foods. These findings are concurrent with the present study, which found no significant differences in muscle activity at 25%, 50% and 75% bite force levels between age

groups, but did find a significant effect of age on the maximal muscle activity in the female cohort. These present findings support the previous research that proposes the masticatory system continues to function at a submaximal level throughout ageing but maximal muscle contractions or bite forces become increasingly reduced. The non-significant difference in muscle activity between the male cohorts was unexpected as muscle strength has been shown to decrease with increasing age in males and females over 50 years. As there were no significant differences in mandibular BMD between the >50yrs males and females, the reason for the rapid decline of maximal muscle activity in females compared to males may again be due to the rapid onset of skeletal changes due to the menopause, compared to the slow and stable decline in males. Other studies, which have reported data from both dentate and edentulous participants have found similar reductions in bite force capabilities; Caloss et al. (2011) found occlusal instability during maximal biting resulted in lower facial muscle activity compared to stable bilateral biting in denture wearers. The reduction in maximal muscle activity due to instability may be reflected in the present over 50yrs cohort, which consisted of 63% mixed dentition of which 11% wore dentures. Furthermore, the present study found significant sex differences in masseter muscle activity but not temporalis muscle activity, in the over 50yrs cohort. The literature reported above used sample groups of pooled men and women and did not report the sex differences in muscle activity.

Correlation results between bite force, muscle activity and BMD values showed that the under 25yrs females cohort exhibited a significant correlation between masseter and temporalis muscle activity, as did the >50yrs females as well as between bite

force and mandibular BMD. The <25yrs males exhibited significant correlations between bite force and masseter muscle activity with no significant correlation between masseter and temporalis muscle activity. The >50yrs males did show a significant correlation between masseter and temporalis muscle activity as well as between bite force and ramus BMD. The <25yrs male findings are concurrent with Ferrario et al. (2004) who reported a linear relationship between bite force and muscle activity in the masseter and temporalis, in young adults. The correlation between masseter muscle and temporalis muscle activity, present in the >50yrs group, may indicate the functionality of the jaw elevator muscles; both muscles were recruited during maximal biting and increased activity in conjunction with one another. However, the bite force- muscle activity correlation is not present in the >50yrs male or female cohorts, this may be confounded by the other facial muscles that are recruited during movement, however the masseter is the most commonly investigated jaw elevator muscle due to its integral role in jaw closing. Instead, bite force significantly correlated with BMD of either the ramus or the mandibular body. These changes may reflect the negative effect of mixed or false dentition on bite force due to the decrease in bite force, without a corresponding decrease in muscle activity. Furthermore, the jaw muscle may require greater activity to stabilise the jaw during biting, which would produce muscle activity that was not converted into an increase in bite force. Previous studies have reported the relationship between muscle activity and bite force in edentulous or older cohorts (Van Der Bilt et al., 2008; Caloss et al., 2011;). Caloss et al. (2011) reported that EMG activity and bite force did not follow the same pattern in denture wearers, when bite force was

higher muscle activity was reduced and vice versa, and where the muscle activity was significantly different, the bite force was not. In a study of 19-69yrs old dentate men and women, Van Der Bilt et al. (2008) reported that average bilateral bite force was 24% larger than unilateral force and muscle activity was higher (960µV) across all four elevator muscles during bilateral biting than unilateral (710µV right, 730µV left). Furthermore, the study reported that the bilateral and unilateral bite forces correlated to bilateral and unilateral muscle activity, as well as a significant negative effect of age on bite force and muscle activity (Van Der Bilt et al., 2008). The present findings are similar to Van Der Bilt et al. (2008) with regards to the negative effect of age on bite force and muscle activity in the female cohort, but did not find a similar decrease in the male cohort. The mixed results in the present study may reflect the mixed dentition in the over 50yrs age group or the rapid age related changes post menopause in females compared to males. However, to the author's knowledge, no previous studies link reduced bite force, age and reduced mandibular BMD, in a single cohort. Reduced bite force has been linked to reductions in mandibular and maxillary bone mineral density in terms of osteoporosis; Siéssere et al. (2009) compared healthy controls to osteoporotic women diagnosed by panoramic radiographs and confirmed by bone densitometry (aged 50-70yrs). They showed significantly lower bite force but significantly higher muscle activity in the jaw elevator muscles during rest and movement but not during maximal biting, in the osteoporotic group. Similar comparisons between BMD, bite force and muscle activity do not appear to have been made in healthy ageing populations.

Bone Mineral Density

There was a significant decrease in BMD between <25yrs and >50yrs female cohorts but this was not replicated in the male cohorts. The >50yrs males did exhibit significantly lower mean mandibular body BMD values than the <25yrs males. A decrease in BMD due to age related changes is expected in bones that are not heavily loaded, such as the mandible, as the effects of ageing cannot be slowed through physical activity/loading. Previous studies using DXA, have focussed on reduced BMD in the mandible and/or maxilla due to age related bone loss or osteoporosis (Horner et al., 1996; Drage et al., 2007; Esfahanizadeh et al., 2013; Geraets et al., 2014). Similarly, Devlin and Horner (2007) reported a reduction in mandibular body and ramus BMD with increasing age in a cohort of 72 females mean age 62yrs. The mean BMD of the mandibular body was 1.15±0.26 g/cm^2, which is slightly higher than that found in the female cohort (0.99±0.41g/cm^2) but equivalent to the male cohort (1.16±0.30g/cm^2) in the present study. The mean BMD of the ramus was not stipulated but from a scatterplot it seemed to range from ~0.25g/cm^2 to ~1.25g/cm^2, in a mixed group of edentulous and dentate women, which is also concurrent with the present study findings (ramus BMD 0.58±.23g/cm^2 females and 0.72±.15g/cm^2 males). Furthermore, their finding was significant regardless of dentition status, which is concurrent with the present study, as is the strong correlation between ramus and mandibular body in the >50yrs female cohort, also reported by Devlin and Horner (2007). However, the results from the present study showed an increase in ramus BMD in the over 50yrs cohort (0.72±0.15g/cm^2 >50yrs

males, 0.58±0.23g/cm^2 >50yrs females) compared to the under 25yrs (0.65±0.26g/cm^2 <25yrs males, 0.55±0.21g/cm^2 <25yrs females) by DXA measurement. This is an unexpected finding, which may be explained by the structural changes occurring in the mandible with age. Such changes have been reported by West and McNamara Jr (1999), to significantly increase in length ($p<0.001$) (from the condyle to angle and from the angle to symphysis) from adolescence to mid-adulthood. Drage et al. (2007) reported that the ramus BMD strongly correlated with the BMD at the hip and lumbar spine and both the ramus and hip exhibited a negative relationship with increasing age within a female cohort. The present findings also found a decrease in femoral neck BMD with age in a female cohort (0.98±0.10g/cm^2 <25yrs, 0.81±0.17g/cm^2 >50yrs females), but did not find a similar decrease in >50yrs males.

Correlations within BMD sites in the present study showed a significant correlation between mandibular BMD and lumbar spine BMD in the <25yrs females and a significant correlation between mandibular BMD and ramus BMD in the >50yrs cohort. The male over 50yrs group exhibited a significant correlation between the mandibular body and the lumbar spine but no significant correlations were found in the <25yrs male cohort. In relation to whole body BMD Looker et al. (2009) reported reductions in BMD at the arm, leg, pelvis and total body with increasing age, which suggests all areas of the skeleton are equally affected by ageing, but lacks specific evidence concerning the craniofacial skeleton. Esfahanizadeh et al. (2013) reported a significant reduction in BMD with musculoskeletal metabolic diseases, which also correlated with increasing age; normal BMD controls were aged 49.21±6.8yrs,

osteopenia were aged 52.54±9.2yrs, osteoporotic participants were aged 63.02±10.3yrs. DXA measurements of the jaw BMD were significantly (p=0.0001) different between the three groups. The mandibular and ramus BMD values presented by Esfahanizadeh et al. (2013) are consistently higher than those of the present study (1.39±0.32g/cm^2 vs 0.99±0.41g/cm^2 for the mandibular body and 0.84±0.22g/cm^2 vs 0.58±0.23g/cm^2 for the mandibular ramus, in females). This may be due to the larger regions of interest during the scan analysis, used by Esfahanizadeh et al., (2013) or may be influenced by the ethnicity difference between Caucasian British and Iranian cohorts. The differences in mandibular BMD findings between the present study and previous research may be due to different positioning technique, analysis technique and/or size of the regions of interest, however the values reported in this study reflect those findings of Horner et al (1996). Similar to Devlin and Horner (2007), Drage, (2007) reported that the number of years edentulous had no significant effect on BMD of the jaw, suggesting that age is a greater effecting factor of facial BMD. This is reflected in the mandibular BMD in the >50yrs female and >50yrs male cohort compared to the <25yrs cohorts. Esfahanizadeh et al., (2013) reported all regions of the jaw (mandibular body, ramus, anterior mandible and anterior maxilla) correlated with hip and lumbar spine BMD. The >50yrs in the present study were largely physically active, which is likely to have helped them maintain BMD at loaded skeletal sites and reduced the speed at which the natural effect of ageing occurs on skeletal muscle. This is reflected in the male cohort, but a large decrease in femoral BMD is still observed in the female cohort, which is likely to reflect the reduction in BMD in post-menopausal women and

higher natural rate of bone loss. However, the lack of correlation between femoral neck and mandibular sites in the over 50yrs group may indicate that the mandible reduces in BMD at a greater rate to the loaded bones, perhaps due to loss of dentition, reduced loading and muscle pull. The lowest mandibular body BMD values were reported in the >50yrs female (0.99±0.41g/cm^2) and >50yrs male cohorts (1.16±0.30g/cm^2), which were 28% and 17% lower than the <25yrs cohorts respectively. These findings indicate an increased risk of facial injury on impact, whilst the lower limb BMD will be maintained through repeated loading. This will add greater risk to Master athletes and those who participate in recreational sport and activity in later life, particularly sports which pose an injury risk to the face. It is recommended that facial protection, ideally custom made, be worn by older athletes in sports that allow it, due to the age related reduction in skeletal integrity.

Conclusion

The existing literature suggests that there is a link between axial and appendicular skeletal BMD measurements with facial BMD, all of which are affected by the onset of ageing. The present study reflects this, but not all skeletal sites significantly correlate. This may be because the cohort of over 50yrs fit into a healthy population who were physically active, rather than exhibiting BMD values indicative of metabolic bone diseases, which form the basis of previous research. This study found that ageing affects bite force, muscle activity and facial BMD in a healthy sample of >50yrs females but not in males. The expected link between maximal bite

force, maximal muscle activity and BMD of the mandible is absent in the younger age cohort, but a correlation between bite force and mandibular BMD (specifically in the mandibular body) is present in the over 50yrs category. In relation to potential facial sports injury, older individuals who exhibit lower facial BMD due to ageing may benefit from customised facial protection, if they partake in high impact sports, which pose a greater risk of facial fracture.

The Subsequent Chapter

The following chapter will focus on the effect of ethnicity on bite force, muscle activity and mandibular BMD, specifically examining the differences between males aged 18-25yrs, which represents the cohort that is most prevalent for sports injuries. Differences in BMD between ethnicities are reported in previous research, but bite force, muscle activity and mandibular BMD differences are under reported.

Chapter 5: The effect of ethnicity on bite force, muscle activity and BMD in a cohort of young adult males.

Introduction

Ethnicity differences in bite force values have not been extensively investigated, the studies available have mainly focussed on single specific ethnicity groups. Furthermore, two large reviews of bite force by Bakke (2006) and Koc et al. (2010) make no mention of ethnicity differences reported in the literature. Nevertheless, Shinogaya et al. (2001) examined differences between young adult men and women of Danish and Japanese origin aged 20-26yrs. Their results showed that Japanese females had significantly higher mean pressure measurements (42.3MPa ±3.8) at the pre-molar and molar regions than Danish females (37.6MPa ±3.5), thus showing a 11% higher mean variance. They also reported variations in maxillary arch width between ethnicities, thus emphasising that variation within craniofacial structures could influence bite force. Regalo et al. (2008) also examined differences in bite force between Indigenous and Caucasian Brazilian Nationals, males and females aged 18-28yrs. They reported a significantly higher mean incisor bite force (206±24N males, 140±20N females) in the Indigenous population when compared to the Caucasian population (150±18N males, 93±15N females) showing a variance of 27% between male cohorts and 33.5% in female cohorts. However, the molar region showed no significant difference (Indigenous 502±47N males, 272±34N females, Caucasian 484±53N males, 288±50N females) (Regalo et al., 2008).

Differences in craniofacial morphology, particularly the size and dimensions of the jaw, between ethnicities (Drummond, 1968; Miyajima et al., 1996; Gu et al., 2011) can subsequently affect facial muscle activity, occlusal contact and bite force in individuals (Gomes et al., 2010; Custodio et al., 2011). For example, Custodio et al. (2011) reported shorter faced individuals exhibited significantly ($p<0.05$) higher maximum bite force (524.5±153.0N short, 389.7±162.8N medium, 272.6±149.1N long) and masseter muscle activity (76.0±5.4%μV short, 75.2±5.6%μV medium, 75.0±3.6%μV long) than medium and long face individuals. Furthermore, the relationship between facial muscle mass, muscle force and bite force may be affected by ethnic differences in craniofacial morphology.

African American men and women, in relation to appendicular measurements, have been reported to have the highest muscle mass, followed by Caucasian, Hispanic and finally the Asian population (Silva et al., 2010). A previous study by Gallagher et al. (1997) also reported that African American women had significantly higher skeletal muscle mass in the leg and the arm compared to Caucasian women, and African American men had significantly higher muscle mass in the arm compared to Caucasian men. In relation to craniofacial research and muscle size Raadsheer et al. (2004) measured bite force, static arm flexion and static leg extension, in a cohort of young adult males and females (aged 18-36yrs, mean 23yrs), of undisclosed ethnicity. The study reported a correlation of jaw muscle size with limb muscle size. However, muscular force (at jaw, arm and leg) differed within individuals and was significantly ($p>0.002$) affected by stature (Raadsheer et al., 2004). This suggests that despite a correlation in muscle size, individuals with a high limb muscle force did not

exhibit an equivalent increase in bite force. Furthermore, the differences in force within tall individuals were not the same as the differences in force within short individuals. These findings suggest that muscular force is affected by stature at the limbs and the jaw, but in different ways, and may confirm or confound muscle force differences between ethnicities. These findings are important because the relationship between muscular force and osteogenic response within bone is key to improving or maintaining the strength of the skeletal system.

Bone Mineral Density

BMD has been reported to be significantly higher in African Caribbean populations compared to Caucasian populations, and this is apparent in children from Tanner stage IV and V (Seeman, 2008), which is usually experienced in mid to late adolescence. BMD continues to be higher in African Caribbean populations at the spine, femur, distal radius and total body throughout adulthood (Ettinger et al., 1997; Looker et al., 2009). Comparisons of BMD between these ethnicities show young adult African Caribbean women have, on average, an 8% higher mean BMD at the lumbar spine than Caucasian women (1.130 g/cm^2 African Caribbean, aged 31.0±3.1yrs; 1.045 g/cm^2 Caucasian, aged 31.8 ±3.1yrs). In addition, African Caribbean women had an 11% higher mean BMD at the femoral neck than Caucasian women (0.962 g/cm^2 and 0.862 g/cm^2 respectively) (Ettinger et al., 1997). Similarly, young adult African Caribbean males exhibit, on average, an 11% higher BMD at the lumbar spine than Caucasian males (1.148 g/cm^2 -1.030 g/cm^2, aged 30.7±3.2yrs).

Additionally, there was a 19% higher mean BMD at the femoral neck in African Caribbean males when comparing the same two groups (1.068 g/cm^2 African Caribbean and 0.891 g/cm^2 Caucasian) (Ettinger et al., 1997). Further evaluation reveals that these differences in bone density between ethnic groups, remains relatively constant in males throughout adulthood (Looker et al., 2009). In relation to ethnicity BMD differences by DXA, studies have often concentrated on key skeletal sites such as the lumbar spine, femur, and distal radius. In contrast, DXA measurement comparisons of the craniofacial skeleton are rare (Ettinger et al., 1997; Looker, 2002; Stone et al., 2003; Looker et al., 2009). Previous measurements of the mandible in comparison to other skeletal sites for the prediction of osteoporosis, have been used in single ethnicity studies. These studies have investigated age ranges from 20–60+yrs but the vast majority focus on 50+yrs (Horner et al., 2002; Drage et al., 2007; Li et al., 2011; Shaw et al., 2012; Esfahanizadeh et al., 2013) or in some circumstances no mention of ethnicity were made (Horner et al., 1996; Kyrgidis et al., 2011). Li et al. (2011) measured a large (111 males, 113 females) cohort of Chinese participants, they reported significant ($p<0.01$) comparisons between mandibular chin BMD and lumbar vertebrae in males and females aged 20-29yrs. In addition, the study reported significant ($p<0.01$) comparisons between mandibular chin BMD and lumbar vertebrae as well as mandibular angle and lumbar vertebrae ($p<0.05$) in males and females aged 30-39yrs. Although these findings are relevant to a Chinese population, they indicate a link between skeletal sites BMD and mandibular BMD in a young adult sample.

Aetiology of Facial injury in Sport

The differences in bone mineral density between ethnicities and younger age groups could potentially have an effect on facial injury outcomes, specifically amongst young adults who participate in sports. The occurrence of facial injury in sport can incur a cost to a person's quality of life, their ability to return to work and a financial cost to the National Health Service (NHS) in the UK. Semi-professional or professional athletes are often advised to rest for 6-8 weeks after maxillofacial fracture, or 3 months if they participate in contact sports, which would result in time away from training and competition (Roccia et al., 2008). In extreme cases significant or complex maxillofacial trauma will require surgical intervention, have significant co-morbidities, have other serious health implications, and even lead to long term health problems (Ai-Ourainy et al., 1991; Covington et al., 1994). The consequences of craniofacial fractures can be as severe as long term brain disorders, loss of brain and cranial nerve function and associated functional and/or aesthetic deficits (Braakman, 1972; Girotto et al., 2001) even 12 months post injury (Coello et al., 2010). Facial injury can have a detrimental effect on a sports person/athletes' long-term health and career, thus the prevalence of facial protection in sport is of high importance.

Facial injuries can be sustained in sport through three possible scenarios: impact with the ground, impact with another player (e.g. clash of heads in football) and impact with equipment (Delilbasi et al., 2004). Impact with another player has been found to cause 43% (Delilbasi et al., 2004) to 63% (Mourouzis and Koumoura, 2005)

of sport related injuries. However, in countries where ball sports are prevalent, impact with equipment, specifically the ball, accounted for 74% of all maxillofacial and skull base fractures (Elhammali et al., 2010).

As many studies are conducted in either European countries (Mourouzis and Koumoura, 2005; Roccia et al., 2008; Elhammali et al., 2010) or the USA (Conn et al., 2003; Erdmann et al., 2008), the most prevalent ethnicity is Caucasian, with Black, Asian, Hispanic and Native Americans forming minority groups. The most common site reported for facial fracture is the mandible (Delilbasi et al., 2004; Roccia et al., 2008), more specify the mandibular angle, followed by mid-face fractures such as the zygoma (Mourouzis and Koumoura, 2005). European studies have reported that the mandible and zygoma are the most prevalent sites susceptible to fracture (Mourouzis and Koumoura, 2005; Roccia et al., 2008; Elhammali et al., 2010), whilst one study conducted in New Zealand reported 41.4% of sports related facial fractures involved the mandible and 29.4% involved the zygoma (Antoun and Lee, 2008). Furthermore, the mandible and zygoma have been identified as the most prevalent sites for facial injury in a study reporting Japanese sports injury rates, however these findings were largely attributable to the prevalence of Baseball in Japan (Delilbasi et al., 2004). Conn et al. (2003) reported Caucasian patients were estimated to sustain 88.4% of all sport and recreational related injuries whilst Black populations only accounted for 6.9%, but the age adjusted injury rate for Caucasians was only 1.5 times higher (28.8 v 19.0 per 1,000 population) than that of Black patients. Data from the Active Peoples Survey conducted in the UK, shows that 34% of 16-24 year old Caucasian British respondents participated in 30 minutes of sport

and/or active recreation 3 times per week. In contrast, within the same survey Black ethnicities reported the lowest participation (~23%) for the same age group (Long et al., 2009). Furthermore, the sex disparity between Black ethnicities was 8.6% in favour of males, whereas Caucasian males were only 4.9% more active than Caucasian females (Long et al., 2009). Conversely, in an American population of 75% Caucasian, 12.3% African American (US Census Bureau, 2000), Burt and Overpeck (2001) reported Caucasian males under 24yrs presented the same hospitalisation rates as Black males under 24yrs, with 20% of all sports related injuries occurring to the face, scalp and neck. However these findings may be affected by the number of injuries sustained in pre-pubescent childhood, before ethnic differences in BMD become apparent, as the 5-14yrs cohort exhibited the greatest number of sport related injuries (Burt and Overpeck, 2001).

Hospital admissions for sports related facial injuries are most prevalent in men in their late teens, into early adulthood (Conn et al., 2003; Delilbasi et al., 2004; Mourouzis and Koumoura, 2005; Elhammali et al., 2010), which highlights a male propensity for sports related facial injury. The highest reported male to female ratio of injury rates is 19:1 (Delilbasi et al., 2004) but 9:1 (Mourouzis and Koumoura, 2005) and 8:1 (Roccia et al., 2008) are commonplace, indicating a higher proportion of young male casualties. These ratios may be influenced by the ratio of male to female participation in sport, which is reportedly lower amongst teenage girls (Vilhjalmsson and Kristjansdottir, 2003) and young adult women (Telama et al., 2005).

The benefits of wearing protective equipment in sport, particularly head protection, have both physical health and financial importance. Yet rules and regulations differ

between sports; In a review of head and face protection in UK sport, Farrington et al. (2012) reported that only boxing, kickboxing, cycling and paintballing have mandatory ruling for the use of protective equipment. The strictest regulations are often applied to youth sport, but senior athletes are often gifted to option of choice in the UK. Other high impact sports such as squash, rugby, cricket and hockey only advise adult players to wear protective equipment (Farrington et al., 2012). Therefore, the differences in facial BMD and muscle activity between ethnic groups may be key to identifying the epidemiological trends in sports related facial fractures and future designs of facial protective equipment. Given this, young adult Caucasian and African Caribbean males present a suitable cohort to measure these outcomes, due to the prevalence of facial sports injuries in that sex and age group.

Aims and Objectives

This study aimed to investigate the effect of ethnicity on bite force, muscle activity and BMD in the mandible, by comparing the differences between Caucasian British and African Caribbean British young adult males aged 18-25 years. The objectives of the study were to;

(i) Identify anatomical BMD differences in both sample groups by DXA analysis.

(ii) Identify whether ethnicity affected bite force, maximal muscle activity and mandibular BMD.

(iii) Investigate how bite force, muscle activity and BMD at all skeletal sites correlated within each sample group.

Materials and Methods

Prior to commencement of the study ethical approval was obtained and granted from the Department of Exercise and Sport Science Research Ethics Committee, Manchester Metropolitan University. The experimental method used in this study follows the procedures described in Chapter 2 Section 2.6 'Materials and Methods'. The cohorts measured within this study are described below.

Participants

Exclusion criteria for the study was a previous history of facial fracture or facial surgery, current or recent orthodontic treatment/surgery, dental treatment within 6 months that consisted of more than a routine check-up (particularly including X-ray or CT scanning), long term parafunctional habits such as bruxism, temporomandibular dysfunction, masticatory pain or diseases that are known to effect bone metabolism. A total of 27 male participants aged 18-25yrs were recruited, which consisted of two groups; (i) 15 Caucasian British males (mean age 20.9±1.7yrs) and 12 African Caribbean British males (mean age 21.5±1.69yrs). Full anthropometric measurements were taken and are shown in Table 5.1. All participants were informed of the study design and procedures and gave written

informed consent prior to taking part in the study. In terms of participation in sport and exercise, the Caucasian group had a slightly greater mean number of hours exercising (4.53 ±0.83hrs) than the African Caribbean group (3.82 ±1.4hrs).

Data Analysis

A one-way MANOVA was performed on the bite force, muscle activity and bone mineral density data (SPSS statistical analysis software 19). Each MANOVA measured the effect of sex on four grouped dependent variables (Table 5.2). The significance level was set to $p < 0.05$ and the univariate significance level was set to $p < 0.0125$ using the Bonferroni correction for four dependent variables and $p < 0.01$ for five dependent variables (Field, 2009). Levene's test for homogeneity (across all conditions) indicated equal variances ($p > 0.05$) for all independent comparisons, therefore the assumption of homogeneity was met. Skewness and Kurtosis analysis indicated that the z-values for all variables were within the ±1.96 range, therefore they did not differ significantly from normality. Furthermore, Pearson's correlation coefficients were calculated between the main dependent variables (maximum bite force, maximum masseter and anterior temporalis muscle activity, BMD at the ramus and the mandibular body) as well as the four BMD measurement sites only (mandibular ramus, mandibular body, femoral neck and lumbar spine). Finally, statistical analyses to determine the differences in fracture rates between the two groups were conducted, using both parametric (t-test) and non-parametric (Mann-Whitney U) tests to compare group means.

Results

The results demonstrated that there were no significant ($p>0.05$) differences between group means of the Caucasian British (CB) and African Caribbean British (ACB) groups in relation to age, height or mass. Therefore, the two cohorts could be regarded as age, height, and mass matched (Table 5.1).

Groups characteristics	Caucasian British (n=15) Mean (SD)	African Caribbean (n=12) Mean (SD)
Age (yrs)	20.93 (±1.71)	21.55 (±1.69)
Height (m)	1.79 (±0.07)	1.79 (±0.08)
Mass (Kg)	83.54 (±13.76)	85.59 (±19.19)

Table 5.1: Age, height and mass mean values for the CB and ACB cohorts.

The results in Table 5.2, show that ethnicity has a significant ($p<0.05$) effect on the bite force, muscle activity and facial BMD grouped variables, however at a univariate level, only the BMD at the ramus was statistically significant ($p<0.0125$). The ACB group exhibited a 33% greater mean bite force than the CB cohort, however statistically this was non-significant ($p>0.05$). Additionally, there was no significant effect of ethnicity on maximal and sub-maximal muscle activity in either the masseter or the temporalis. A significant effect ($p<0.05$) existed in the BMD grouped variables, but on a univariate level, only the ramus was significantly ($p<0.0125$) effected whilst the femoral neck and lumbar spine were approaching univariate significance ($p<0.05$) (Table 5.2).

	Caucasian British Males	African Caribbean Males	*n*	MANOVA *F*	Observed Power	Univariate *F*	P
Main effects	Mean (SD) n=15	Mean (SD) n=12	27	3.337	0.753		*
Max Bite Force (N)	295.3 (±142.6)	393.1 (±165.4)				2.72	ns
Max Masseter (%)	711.3 (±414.4)	615.4 (±281.2)				0.47	ns
Max Temporalis (%)	609.7 (±415.7)	440.0 (±157.6)				1.78	ns
BMD ramus (g/cm^2)	0.65 (±0.27)	0.92 (±0.24)				7.56	†
EMG Masseter			27	0.841	0.225		ns
Max Masseter (%)	711.3 (±414.4)	615.4 (±281.2)				0.47	ns
75% Masseter (%)	577.2 (±287.8)	562.6 (±247.7)				0.02	ns
50% Masseter (%)	471.9 (±289.4)	366.8 (±118.3)				1.39	ns
25% Masseter (%)	285.9 (±158.8)	241.4 (±91.1)				0.74	ns
EMG Temporalis			27	0.969	0.256		ns
Max Temporalis (%)	609.7 (±415.7)	440.0 (±157.6)				1.78	ns
75% Temporalis (%)	494.9 (±323.9)	404.9 (±155.1)				0.83	ns
50% Temporalis (%)	368.3 (±229.2)	276.8 (±103.7)				1.63	ns
25% Temporalis (%)	231.5 (±147.5)	173.1 (±47.3)				1.73	ns
BMD effects			27	3.752	0.807		*
Ramus (g/cm^2)	0.65 (±0.27)	0.92 (±0.24)				7.56	†
Mandible (g/cm^2)	1.40 (±0.29)	1.45 (±0.29)				0.22	ns
Femoral neck (g/cm^2)	1.14 (±0.30)	1.39 (±0.18)				6.75	*
Lumbar spine (g/cm^2)	0.90 (±0.23)	1.10 (±0.21)				5.18	*

Table 5.2: MANOVA results for grouped dependent variables for the CB and ACB cohorts.

(* p< 0.05, **** p< 0.01, **†**Significant using Bonferroni correction for MANOVA with 4 dependent variables (p<0.0125) or with 5 dependent variables (p<0.01))

Pearson's Correlation Coefficient					
CB	Max Bite Force (N)	Max Masseter (%)	Max Temporalis (%)	BMD ramus (g/cm^2)	BMD mandible (g/cm^2)
Max Bite Force (N)	-	-	-	-	-
Max Masseter (%)	0.59*	-	-	-	-
Max Temporalis (%)	0.03	0.50	-	-	-
BMD ramus (g/cm^2)	-0.10	0.02	0.33	-	-
BMD mandible (g/cm^2)	-0.16	0.22	0.25	0.32	-
ACB					
Max Bite Force (N)	-	-	-	-	-
Max Masseter (%)	0.76**	-	-	-	-
Max Temporalis (%)	0.46	0.80**	-	-	-
BMD ramus (g/cm^2)	0.57	0.40	0.07	-	-
BMD mandible (g/cm^2)	0.27	0.22	0.00	0.64*	-

Table 5.3: Correlations between bite force, muscle activity and BMD for the CB and ACB cohorts.

(* $p < 0.05$, ** $p < 0.01$).

A significant ($p<0.05$) correlation existed between the maximal bite force and maximal masseter muscle activity in the CB group (Table 5.3). Significant correlations also existed between maximal bite force and maximal masseter muscle activity ($p<0.01$), maximal masseter muscle activity and maximal temporalis muscle activity ($p<0.01$) and BMD at the ramus and BMD at the mandible ($p<0.05$) in the ACB cohort (Table 5.3).

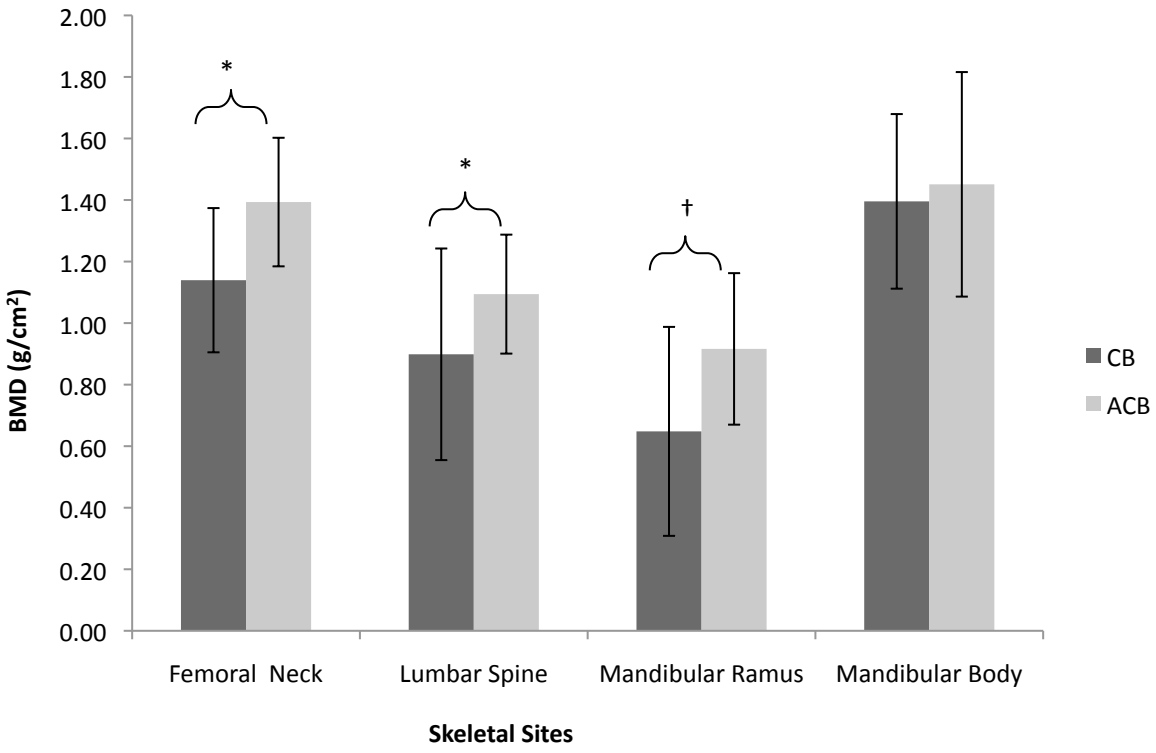


Figure 5.1: BMD values for lumbar spine, femoral neck, mandibular body and ramus for the CB and ACB cohorts.

(*p<0.05, †p<0.0125).

Across all four skeletal sites, ACB males exhibited consistently higher BMD than CB males (Figure 5.1). Specifically they were 22% higher at the femoral neck, 21% higher at the lumbar spine, 41% higher at the mandibular ramus and 4% higher at the mandibular body. Between the groups, significant differences were observed at the femoral neck (p=0.016), lumbar spine (p=0.033) and mandibular ramus (p=0.011).

Pearson's Correlation Coefficient				
CB	BMD ramus (g/cm^2)	BMD mandible (g/cm^2)	BMD femoral neck (g/cm^2)	BMD lumbar spine (g/cm^2)
BMD ramus (g/cm^2)	-	-	-	-
BMD mandible (g/cm^2)	0.33	-	-	-
BMD femoral neck (g/cm^2)	0.02	0.24	-	-
BMD lumbar spine (g/cm^2)	0.39	0.16	-0.03	-
AC				
BMD ramus (g/cm^2)	-	-	-	-
BMD mandible (g/cm^2)	0.64*	-	-	-
BMD femoral neck (g/cm^2)	0.30	0.34	-	-
BMD lumbar spine (g/cm^2)	0.07	-0.14	0.38	-

Table 5.4: Correlations between BMD at skeletal sites for the CB and ACB cohorts Coefficients for BMD variables.

(* $p< 0.05$, ** $p< 0.01$)

No significant correlations were identified between BMD at skeletal sites within the CB cohort (Table 5.4), but there was a significant correlation between mandibular ramus and mandibular body ($p<0.05$) within the ACB group. Each participant completed a questionnaire, which highlighted the number of fractures that they had previously encompassed. The total percentage of fractures (at any skeletal site) per sample group were (53% CB and 27% ACB) not statistically significant ($p>0.05$). In addition, there was no correlation between BMD at any skeletal site and fractures within either group or across groups ($p>0.05$).

Discussion

This study aimed to investigate the effect of ethnicity on bite force, jaw elevator muscle activity and BMD in the mandible, in particular to identify differences between Caucasian British and African Caribbean British young adult males in height, mass, bite force, masseter and temporalis muscle activity and mandibular BMD. In addition, this study aimed to identify whether BMD at all skeletal sites correlated within each sample group and/or correlated with fractures sustained within sample groups. The sample groups were matched on sex, age, height and mass, which suggests that any differences found between the two groups were not attributable to whole body anatomical differences, but highly likely to be attributable to craniofacial differences in ethnicity. Ethnicity had a significant effect on bite force, jaw elevator muscle activity and mandibular BMD when grouped, but this was only significant for the BMD at the ramus when calculated independently.

Bite force and Muscle Activity

The present findings suggest that there are differences between Caucasian and African Caribbean males in mean bite force (295±142N Caucasian, 393±165N African Caribbean) and mean muscle activity (masseter 711±141%, 615±281% and temporalis 606±415%, 440±157% in the Caucasian and African Caribbean groups respectively) but the differences were not large enough to produce significant results. Raadsheer et al. (1999) reported the thickness of the masseter muscle as a

determinant of maximal bite force, in a cohort of undisclosed ethnicity, accounting for a larger amount of variation in bite force magnitude than all other craniofacial measurements. Differences in muscle size between ethnicities has been documented (Wagner and Heyward, 2000), but to the author's knowledge no other paper has reported differences the craniofacial muscle activity between ethnicities. The effect of muscle thickness on bite force does not directly relate to the effect of muscle activity on bite force, but it may indicate a relationship between craniofacial muscle and bite force. A review of bite force literature by Bakke (2006) highlighted the effect of facial morphology on the performance of the mandible as a lever, taking into account facial dimensions, angles and occlusal contact area. Custodio et al. (2011) reported shorter faced individuals exhibited significantly higher maximum bite force and masseter muscle activity than medium and long face individuals. Although these findings were of individuals of the same ethnicity rather than Caucasian versus African Caribbean, this indicates that the existing differences in facial dimensions between ethnicities may have an effect on the bite force and muscle activity. The significant differences were only partially replicated in the temporalis muscle between the extremes of short and long facial height and similar to Custodio et al. (2011), the strong correlations between masseter muscle activity and bite force reported in both ethnic groups in the present study, was not replicated in the temporalis muscle. This may indicate that the temporalis is less sensitive to facial dimensions, that temporalis muscle force peaks at increased mouth opening (Paphangkorakit and Osborn, 1997) or that the influence of the temporalis muscle on bite force is surpassed by the influence of the craniofacial

morphology. Differences in facial morphology and size of dentition between Caucasian and Black groups may influence the aptitude of the mandible as a lever and thereby affect the loading of the mandible, which would influence the mandibular BMD. This may indicate prevalence for stronger bite forces in one particular population, however there are no bite force studies between on African Caribbean participants to confirm these differences. In relation to the EMG findings, the present study found no significant effect of ethnicity on muscle activity in either the masseter or the temporalis; this is true for both sub-maximal and maximal bite force. Although jaw muscle size has been found to correlate with limb muscle size in young adults (Rassdsheer et al., 2004), their corresponding muscular forces measured during maximal bite force did not correlate to forces during arm flexion or leg extension in the same way amongst all individuals (Raadsheer et al., 2004). This may indicate that the differences in muscle force are confounded by the complex differences in muscle groups or facial morphology and therefore do not directly translate into differences in bite force. Moreover, activity in the masseter and temporalis muscles may under represent the additional activity provided by other masticatory muscles, such as the medial and lateral pterygoids. Further studies relating differences in muscle mass, size or thickness to ethnicity were most commonly anthropometric based and did not include muscle strength or EMG muscle activity as a parameter (Gallagher et al., 1997; Wagner and Heyward, 2000; Silva et al., 2010). Differences in muscle strength are most commonly reported in relation to the appendicular skeleton, in the ageing population (Hughes et al., 2001).

Bone Mineral Density

In the present study, only the BMD of ramus (0.65±0.27g/cm^2 Caucasian, 0.92±0.24g/cm^2 African Caribbean) was significantly affected by ethnicity when variables were grouped. However both the BMD of the lumbar spine (0.90±0.23g/cm^2 Caucasian, 1.10±0.21g/cm^2 African Caribbean) and femoral neck (1.14±0.30g/cm^2 Caucasian, 1.39±0.18g/cm^2 African Caribbean) were significantly different between ethnicities when considered separately, showing higher values within the African Caribbean group. Differences in skeletal muscle mass and bone mass at both the upper and lower extremities have been found between white and black populations in their mid to late 40s. Black males most commonly exhibit greater arm muscle mass and ratio of limb bone length to height, despite being matched for weight (Gallagher et al., 1997). Similarly, Ortiz et al. (1992) reported lower skeletal muscle mass and regional bone mineral at the upper extremities in Caucasian women compared to Black women. Similar significant differences were also reported for the lower extremities and total appendicular skeletal muscle and regional bone mineral between the two ethnicities, when matched for age, height, weight and menstrual status (Ortiz et al., 1992). These findings are further supported by Wagner and Heyward (2000), who reviewed body composition studies between Black and White populations. Wagner and Heyward (2000) identified definitive differences in bone mass, bone mineral density and bone mineral content which were consistently greater in black populations. Additional studies have found black male populations to have consistently higher BMD at skeletal sites than Caucasian

males (Nelson et al., 1995; Ettinger et al., 1997; Wang et al., 1997; Looker et al., 2009). Nelson et al. (1995) who reported 5% greater BMD at the radius, 10% at the lumbar spine and 20% at the femoral neck in Black men compared to Caucasian men, with no significant anthropometric differences. These findings are corroborated by Ettinger et al. (1997) who reported young Black men exhibited on average, a 12% greater BMD at the lumbar spine and a 20% greater BMD at the femoral neck compared to young Caucasian men. In terms of femoral neck and lumbar spine BMD, these findings are concurrent with the present study, which found African Caribbean males exhibited a 22% greater BMD at the femoral neck and a 21% greater BMD in the lumbar spine, than Caucasian males. This study also reported 41% greater BMD at the mandibular ramus in the African Caribbean cohort compared to the Caucasian cohort. A study by Ong and Stevenson (1999) found Australian males and females of Asian descent had 20% higher BMD at the mandibular angle than those of Caucasian decent, using measurements from radiographs. Although the ethnic groups are different to the present study, it does indicate a strong significant difference in mandibular angle BMD between ethnicities, which is concurrent with the present study. Other studies that measured similar age ranges or ethnicities to the present study, did not report values for mandibular BMD, which indicates these findings as novel to this research study. Nelson et al. (1995) reported no significant differences in fracture experience between ethnicities, which is also in agreement with the present study findings. However, Conn et al. (2003) reported Caucasian patients were estimated to sustain 88.4% of all sport and recreational activity related injuries, whilst Black populations only accounted for 6.9%. The age adjusted injury rate for

Caucasians, reported by Conn et al., (2003) was only 1.5 times higher (28.8 v 19.0 per 1,000 population) than that of Black patients. With this in mind, these results show mandibular bone density in Caucasian males, as low as 0.26g/cm^2 at the ramus in some participants, which could place these individuals at greater risk of injury. This cohort design is important, as most professional or semi-professional athletes, where facial sports injury is the most prevalent, are within this age range. Athletes with low facial BMD may be at greater risk of fracture, which enforces the need for customised facial protective equipment to be used in sport, to reduce the risk of serious injury.

There were no correlations between BMD at different skeletal sites in the Caucasian cohort, but there was a significant correlation between the mandibular body and the ramus BMD in the African Caribbean cohort. Previous research has reported correlations between the face and loaded skeletal sites in older or edentulous populations (Lindh et al., 2004; Drage et al., 2007; Esfahanizadeh et al., 2013) but little has been reported for young adult and/or multiracial cohorts. Li et al. (2011) reported significant ($p<0.01$) comparisons between mandibular chin BMD and lumbar vertebrae in males and females aged 20-29yrs, as well as significant comparisons between mandibular chin BMD and lumbar vertebrae and mandibular angle and lumbar vertebrae ($p<0.05$), in males and females aged 30-39yrs. Nevertheless, these studies were conducted on a Chinese cohort and although they do indicate a strong correlation between BMD at facial and other key skeletal sites, they may not be applicable to the present study sample groups, based on differences in age and ethnicity. In contrast, Sinaki et al. (1998) investigated site specific changes

in BMD at the lumbar spine and proximal femur to determine whether they correlated with muscle strength, in premenopausal Caucasian women. Although there were some correlations between BMD and muscle strength, they were not site specific, which lead Sinaki et al. (1998) to conclude that positive effects of muscle strength on BMD may be systemic rather than site specific. The absence of correlation between skeletal sites in young adult cohorts may be due to the variation in pre-peak bone mass values or continued development of the facial morphology in young adults (West and McNamara Jr et al., 1999). The lack of correlation between skeletal sites in the findings of the present study, suggest that even individuals with greater hip and lumbar spine BMD may exhibit lower facial BMD values. These findings are important in relation to facial sports injury prevention; individuals with lower facial BMD may be more susceptible if (i) they don't wear protective equipment, (ii) their facial equipment is substandard or non-custom made (iii) they partake in sports where low body weight (e.g. low weight boxers) but high impacts are prevalent.

Conclusion

The present study found a significant difference in BMD at the ramus, femoral neck and lumbar spine between the different ethnic groups, but did not find significant differences in the mandibular body BMD, bite force or muscle activity. Furthermore, the group correlations showed no significant relationships except for that of the mandibular body and ramus in the African Caribbean cohort. The facial dimensions

of the African Caribbean cohort may influence the correlation between BMD at different sites on the mandible, or they may be influenced by a whole-body effect on BMD. The lack of difference in masseter and temporalis muscle activity and bite force, may be due to small and uneven sample sizes (15 CB, 12 ACB). The variables that showed positive differences between the two groups but were non-significant, such as bite force and temporalis muscle activity, may reach significance with an equal number of African Caribbean males in the group.

The Subsequent Chapter

Furthermore, the differences in ethnicity may be linked to facial dimensions, which have been associated with muscle force and bite mechanics. The following chapter will focus on the effect of facial dimensions on bite force, muscle activity and BMD.

Chapter 6: The Influence of Craniofacial Dimensions on Bite force, Muscle activity and Mandibular BMD.

Introduction

The effect of Sex

Lateral cephalometric radiographs are the most frequently used method for analysing craniofacial dimensions in relation to facial/cranial morphology. Sexual dimorphism in relation to facial dimensions is apparent across a number of ethnicities including Caucasian, Japanese, Chinese and Black populations. There are some obvious examples that have been reported and in relation to sex, Japanese females exhibit a steeper mandibular plane angle (26.1±5.4°) in comparison to their male (22.3±3.9°) counterparts (Miyajima et al., 1996). In addition, Japanese males aged 20-25yrs have been found to demonstrate a significantly greater ($p<0.01$) mean mandibular length, measured from the condyle to the gnathion on the chin (125.5±5.1mm) than Japanese females (118.8±4.7mm). This was also found in a European-American cohort of young adult (mean age 36±10yrs) males (132.3±6.8mm) and females (120.2±5.3mm) (Miyajima et al., 1996). Gu et al. (2011) also reported greater mean mandibular length from the condyle to the gnathion in males (125.4±6.9mm Chinese, 136.7±9.9mm Caucasian) compared to females (119.4±7.9 Chinese, 123.0±10.0mm Caucasian). Similarly, the study reported significant sexual dimorphism in the Chinese and the Caucasian cohorts in relation to lower facial height, males expressed significantly ($p<0.001$) greater lower facial

height (74.9mm Chinese, 71.7mm Caucasian) than females (69.4mm Chinese, 65.0mm Caucasian) within the same ethnicity. Franklin et al. (2008) examined sexual dimorphism in Black South Africans, they measured nine anatomical sites on cadaveric mandibles. They reported that all sites (ramus height, symphysis height, coronoid height, bi-gonion breadth, bi-condylar breadth, symphysis breadth, bi-coronoid breadth, corpus length, and maximum mandibular length), were significantly different between males and females. However, coronoid height (58.5±4.7mm males, 52.5±3.7mm females, $F=113.3$, $p<0.001$), ramus height (56.7±4.9mm males, 51.0±3.7mm females, $F=94.2$, $p<0.001$) and mandibular length (120.9±4.7mm males, 114.9±4.7mm females, $F=92.9$, $p<0.001$) were the greatest predictors of sexual dimorphism.

The effect of Age

Changes to the material properties of the human skeleton, such as bone mineral density and cortical thickness, are slow throughout adulthood (Seeman, 2004; Blain et al., 2008). Large or dramatic changes only occur through immobility, disease or most commonly menopause in females (Christiansen et al., 1987; Frost, 2001; Blain et al., 2008). Research suggests that the craniofacial skeleton appears to continue to grow throughout adulthood and even into old age (Israel, 1973; Doual et al., 1997; West and McNamara Jr, 1999). West and McNamara Jr (1999) reported that linear cephalometric measurements increased significantly over time; these included the anterior facial height (131.0±9.0mm to 135.6±8.8mm $P\leq0.001$), ramus height

(64.6±5.5mm to 68.4±5.5mm, p≤0.001) and mandibular length from ramus angle to chin (82.4±4.6mm to 84.0±5.0, p≤0.001). However, angular measurements did not significantly change in males or females over time; from adolescence to early adulthood females exhibited a slight posterior rotation of the mandible whilst males experienced a small anterior rotation of the mandible. However, this did not continue into mid adulthood and was not was not significant (West and McNamara Jr, 1999).

The effect of Ethnicity

As well as sex and age, ethnicity has been found to affect the dimensions of the face; Miyajima et al. (1996) compared cephalometric measurements of young adult Japanese (aged 20-25yrs) and European-Americans (aged 30±10yrs). They reported that Japanese men and women had smaller mandibular lengths, (measured from the condyle to the gnathion on the chin), (125.5±5.1mm males and 118.8±4.7mm females) than European-American (132.3±6.8mm males and 120.2±5.3mm females). Similarly, Gu et al. (2011) also reported a significantly smaller mandibular length in Chinese males compared to Caucasian males (125.4±6.9mm Chinese, 136.7±9.9mm Caucasian, p<0.001) but this difference was not replicated in Chinese females (119.4±7.9 Chinese, 123.0±10.0mm Caucasian, p>0.05). Furthermore, Gu et al. (2011) found Chinese females had a significantly (p<0.001) greater lower facial height (69.4±6.2mm Chinese) compared to the Caucasian females (65.0±6.9mm Caucasian), but this difference was not replicated in the male cohorts. In addition,

Bacon et al. (1983) reported Cameroon African adult males aged 20-30yrs showed a greater facial convexity, greater lower facial height (77.8mm vs 71.0mm) and a smaller upper facial height (53.3mm vs 57.1mm) compared to their male Caucasian counterparts. Freitas et al. (2010) measured 50 Caucasian Brazilians (25 males, 25 female) aged 13.17±1.07yrs, compared to 56 Black Brazilians (28 males, 28 females) aged 13.24±0.56yrs. They reported significant differences between the ethnicities in relation to mandibular length (condyle to gnathion) (110.97±5.41 Caucasian, 108.61±5.97 Black, $p=0.036$), facial convexity (4.60±4.89° Caucasian, 8.47±4.88° Black, $p\leq0.001$) and SN-GoGn angle, which is a measure of lower facial height in angular form (33.01±3.98° Caucasians, 30.54±4.42° Blacks, $p<0.003$). Similarly, Janson et al. (2011) measured a group of 40 Caucasians (20 males, 20 females) aged 13.02yrs and a group of 40 Afro-Caucasians (20 males, 20 females) aged 13.02yrs. When pooled into two ethnic groups, the Caucasian subjects had significantly ($p<0.05$) greater (50.61±2.03mm) upper anterior facial height compared to the Afro-Caucasian group (48.14±2.65mm), but no significant difference was found for lower anterior facial height (59.97±4.89mm Caucasian, 60.49±3.89mm, Afro-Caucasian, $p>0.05$).

Craniofacial morphology may be the key to the differences in bite force, muscle activity and mandibular BMD between individuals. Raadsheer et al. (1999) reported that craniofacial morphology (measured from lateral radiographs) explained 58% of the variance within bite force in a cohort of Caucasian adults, regardless of sex. Furthermore, Braun et al. (1995b) reported that maximum bite force was affected by mandibular plane angle as well as posterior facial height. Similarly, Van Spronsen et

al. (1997) linked differences in morphology to the spatial orientation of the jaw elevator muscles, they reported that anterior facial height significantly correlated with jaw muscle orientation. In terms of mechanical levers, the length and angle of the mandible will affect the movement pattern and muscular force needed to open and close the jaw (Van Spronsen et al., 1997; Van Eijden, 2000) which may in turn have an effect on the BMD of the mandible. In addition, Hara et al. (2010) suggested craniofacial morphology affected the fatigability of the masseter muscle; greater palatial plane angel, anterior facial height and molar height caused early onset of fatigue in a Japanese cohort. Furthermore, Kohakura et al. (1997) measured the relationship between cephalometric measurements of cadaveric skulls and cortical bone thickness of the mandible, at the lower incisor, premolar and 1^{st} and 2^{nd} molars. The results showed that the Gonion-Gnathion (mandibular length, angle to menton) significantly correlated with buccal cortical bone thickness at the premolar ($r=.49$, $p<0.01$) and 1^{st} molar ($r=.37$, $p<0.05$) and the ramus height (condyle to gonial angle) significantly correlated with buccal cortical bone thickness at the premolar ($r=.42$, $p<0.01$). Furthermore, the ramus width significantly correlated with buccal cortical bone thickness at the premolar ($r=.48$, $p<0.01$) and 1^{st} molar ($r=.33$, $p<0.05$). Therefore, differences in craniofacial morphology can affect masticatory function, occlusal contact area, bite force and masticatory muscle activity, as well as a potential effect on the material properties of bone (Kohakura et al., 1997; Gomes et al., 2010; Custodio et al., 2011). Differences in the biomechanical performance of the jaw may thereby affect the relationship between bite force, muscle activity and BMD of the mandible, regardless of sex, age or ethnicity.

Aims and Objectives

The aim of the present research study was to investigate how facial dimensions relate to bite force, muscle activity and BMD in the mandible, in a cohort that encompasses different sexs, ages and ethnicities. The objectives of the study were to;

(i) Identify how the sample groups differ in terms of facial dimensions.

(ii) Identify which facial dimension variables correlated with bite force, muscle activity and/or mandibular BMD.

(iii) Identify to what extent each of the facial dimension variables explained the variation in bite force, muscle activity or mandibular BMD.

Materials and Methods

Prior to commencement of the study ethical approval was obtained and granted from the Department of Exercise and Sport Science Research Ethics Committee, Manchester Metropolitan University. The methodological procedures in this study are described in Chapter 2 Section '2.6 Materials and Methods'.

Participants

Inclusion criteria for the study was male and female participants aged 18-25yrs or over 50yrs, of Caucasian ethnicity or male participants aged 18-25yrs of African Caribbean ethnicity. Exclusion criteria for the study was a previous history of facial fracture or facial surgery, current or recent orthodontic treatment/surgery, dental treatment within 6 months that consisted of more than a routine check-up (particularly including X-ray or CT scanning), long term parafunctional habits such as bruxism, temporomandibular dysfunction, masticatory pain or diseases that are known to effect bone metabolism. A total of 72 participants were analysed who had been included in the previous research studies. The cohort consisted of five groups;

(i) 15 Caucasian British males aged 18-25yrs (mean age 20.9±1.7yrs)

(ii) 15 Caucasian British females aged 18-25yrs (mean age 21.3±2.0yrs)

(iii) 15 Caucasian British Males aged 50+yrs (mean age 63.5±7.3ys)

(iv) 15 Caucasian British females aged 50+yrs (mean age 62.4±7.1yrs)

(v) 12 African Caribbean British males aged 18-25yrs (mean age 21.5±1.69yrs).

All participants were informed of the study design and procedures and gave written informed consent prior to taking part as per the previous studies.

Measurements of the facial dimension variables Upper facial height (UFH), Lower facial height (LFH), Condyle-Angle (C-A), Angle-Menton (A-M), Condyle-Condyle (C-C) and Condyle-Angle-Menton (C-A-M) were made using ImagJ software from lateral photographs as shown in Figure 2.32 (as described in Chapter 2, Section 2.6) and Figure 6.1 and 6.2.

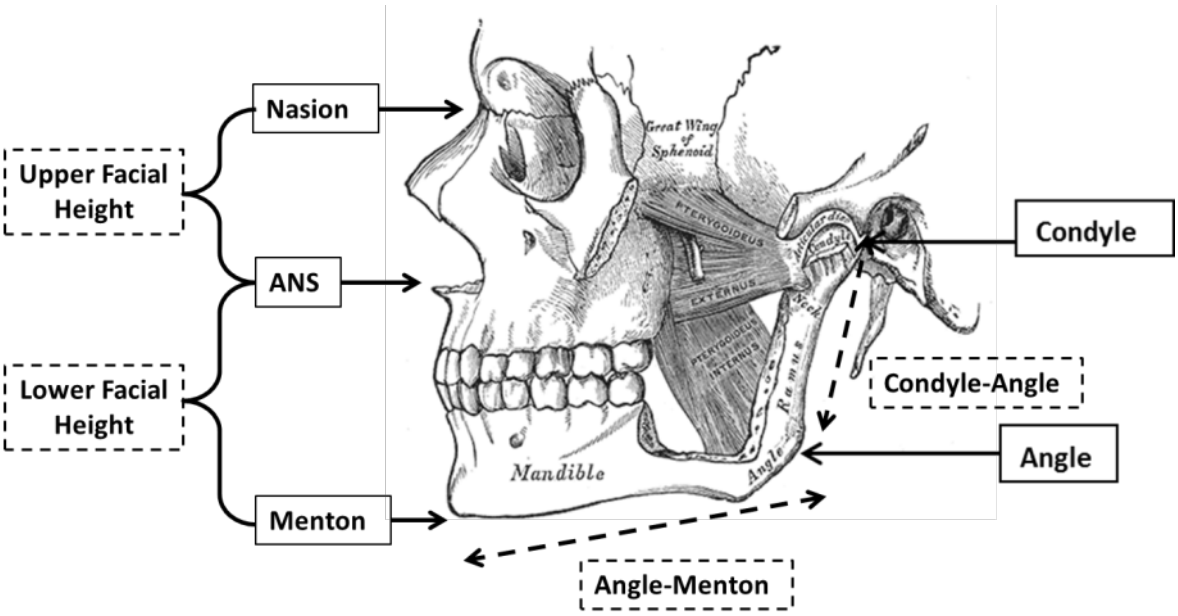


Figure 6.1: Measurement points used for obtaining craniofacial dimensions.

(Original figure obtained from Gray (2010))

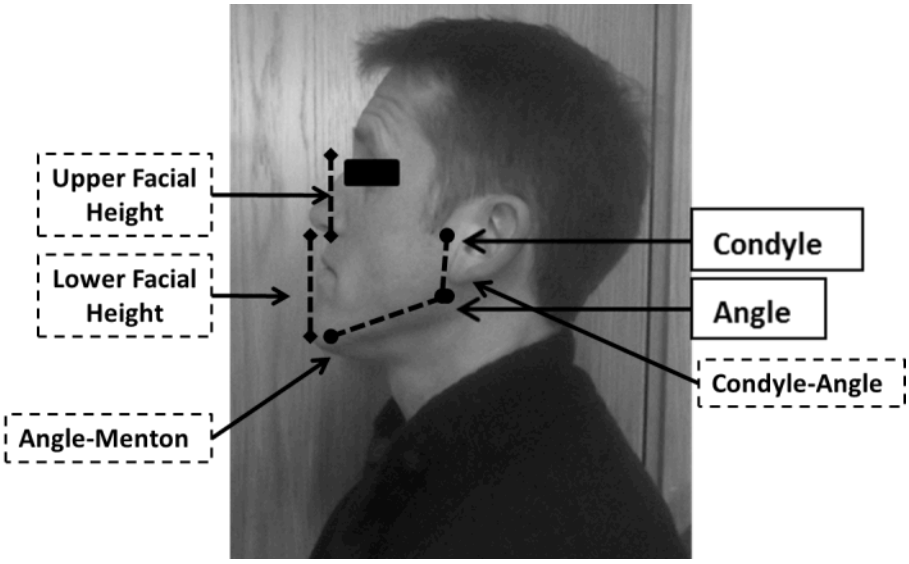


Figure 6.2: Measurement points used for obtaining craniofacial dimensions from lateral photographs.

Data Analysis

A one-way ANOVA was performed on each of the facial dimension variables (UFH, LFH, C-A, A-M, C-C and C-A-M) (SPSS statistical analysis software [IBM SPSS Statistics 19]). Each ANOVA measured the effect of the sample group (ethnicity, sex, and age) on the given facial dimension, with a Tukey's post hoc test. The significance level was set to $p < 0.05$. Conversely, the following analyses measured the effect of facial dimensions on the outcome variables (Bite force, masseter and temporalis muscle activity, ramus and mandibular body BMD), to ascertain whether facial dimensions influence bite force, muscle activity and mandibular BMD regardless of sex, age and ethnicity. Pearson's correlation coefficients were calculated between the facial dimension variables (UFH, LFH, C-A, A-M, C-C and C-A-M) and the main outcome

variables (Bite force, masseter muscle activity, temporalis muscle activity, ramus BMD and mandibular body BMD). Finally, stepwise regression analysis (SPSS statistical analysis software [IBM SPSS Statistics 19]) were conducted on the main outcome variables (Bite force, masseter muscle activity, temporalis muscle activity, ramus BMD and mandibular body BMD) using the facial dimension variables (UFH, LFH, C-A, A-M, C-C and C-A-M) as predictors (SPSS statistical analysis software 19). Levene's test for homogeneity (across all conditions) indicated equal variances ($p>0.05$) for all independent comparisons, therefore the assumption of homogeneity was met. Skewness and Kurtosis analysis indicated that the z-values for all variables were within the ±1.96 range, therefore they did not differ significantly from normality.

Results

Figure 6.3 and 6.4 show the mean and standard deviation of each facial dimension variable for each cohort. The ANOVA results for the effect of sample group on each of the facial dimensions show UFH ($F(4,67)=1.32$, $p>0.05$) and A–M length ($F(4,67)=1.73$, $p>0.05$) were not significantly affected by sample group. Conversely, LFH ($F(4,67)=10.14$, $p<0.001$), C-A-M angle ($F(4,67)=3.36$ $p<0.05$), C–A length ($F(4,67)=5.04$, $p<0.01$) and C-C width ($F(4,67)=7.47$, $p<0.001$) were significantly affected by sample group.

Furthermore, the post hoc tests showed Lower facial height was significantly higher in African Caribbean males compared to <25yrs females ($p<0.0001$), <25yrs males

(p=0.026) and >50yrs females (p<0.0001), it was also significantly higher in <25yrs males than <25yrs females (p=0.01) and >50yrs females (p=0.025). Lower facial height was also significantly higher in >50yrs males compared to <25yrs females (p=0.0003) and <25yrs males (p=0.025). The African Caribbean group had significantly higher C-A-M angle than <25yrs females (p=0.002), <25yrs males (p=0.02), >50yrs females (p=0.01) but was not significantly greater than >50yrs males (p=0.07). The C-A length was significantly higher in <25yrs males compared to <25yrs females (p=0.007), >50yrs females (p=0.0001) and African Caribbean males (p=0.03). The >50yrs females exhibited significantly shorter values for C-A compared to >50yrs males (p=0.02) and African Caribbean males (p=0.011). Finally, the C-C measurements were significantly lower in the <25yrs females than <25yrs males (p=0.0003), >50yrs females (p=0.018), >50yrs males (p<0.0001) and African Caribbean males (p=0.03). The >50yrs males exhibited higher C-C width than <25yrs males (p=0.013) and >50yrs females (p=0.001) but was not significantly greater than African Caribbean males (p=0.07).

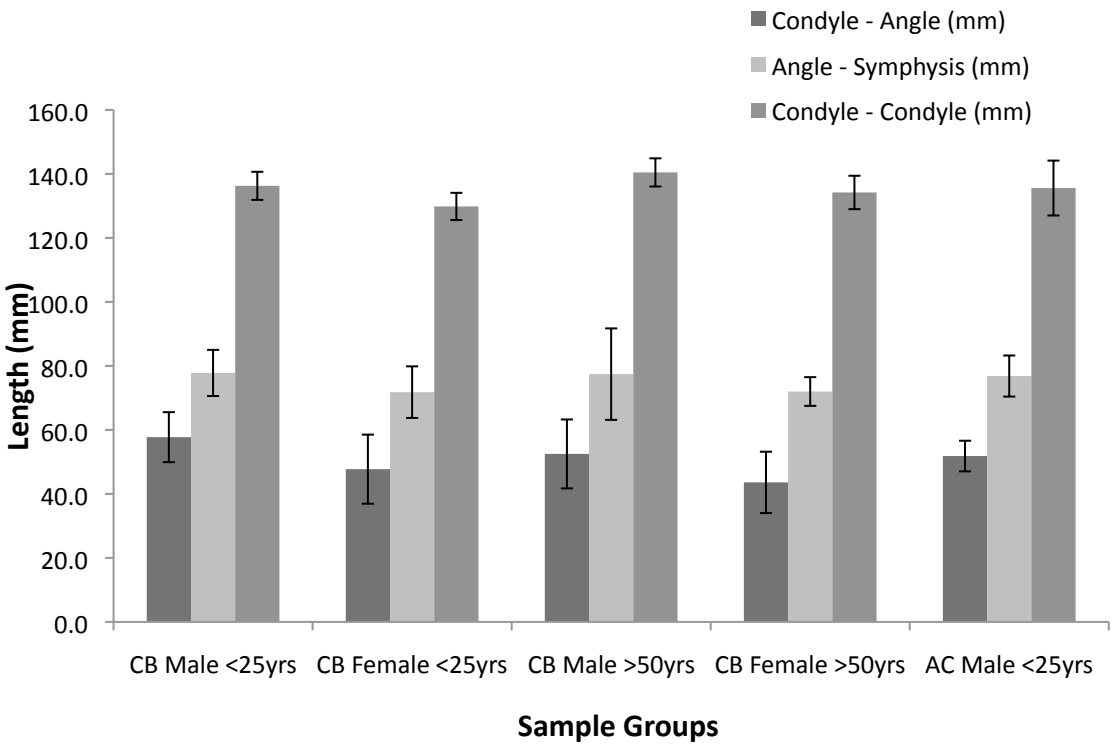


Figure 6.3: Group means and standard deviations for the mandibular measurements for each cohort.

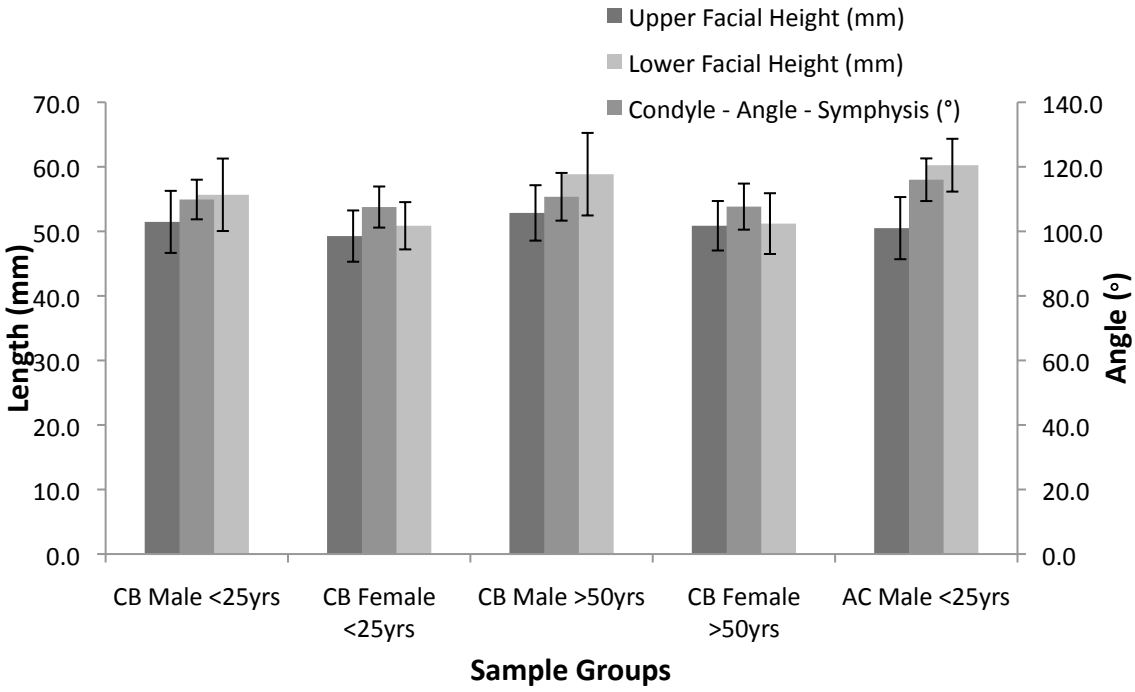


Figure 6.4: Group means and standard deviations for the facial heights and mandibular angle for each cohort.

(Correlation with Maximal Bite Force)	Pearson's Correlation Coefficient	One-tailed Significance
Upper Facial Height (UFH)	0.011	0.465
Lower Facial Height (LFH)	0.137	0.128
Condyle to Angle (C-A)	0.271	0.011*
Angle to Menton (A-M)	0.215	0.036*
Condyle to Angle to Menton (C-A-M)	0.046	0.352
Condyle to Condyle (C-C)	0.149	0.108

Table 6.1: Correlation Coefficients results for maximal bite force in relation to facial dimensions.
(*p<0.05, **p<0.01, †p<0.001)

Max. Bite Force	Model Summary				ANOVA		Standardised coefficients	
	R square	R square Change	F Change	Sig. F Change	F	Sig.	Beta	Sig.
UFH (mm)	0.000	0.000	0.008	0.930	0.008	0.930	-.014	0.910
+ LFH (mm)	0.032	0.032	2.244	0.139	1.126	0.330	0.274	0.095
+ C-A (mm)	0.104	0.072	5.378	0.023*	2.592	0.060	0.304	0.047*
+ A-M (mm)	0.182	0.078	6.257	0.015*	3.660	0.009**	0.454	0.010**
+ C-A-M (°)	0.183	0.001	0.099	0.754	2.908	0.020*	0.130	0.474
+ C-C (mm)	0.190	0.007	0.576	0.451	2.504	0.031*	0.061	0.630

Table 6.2: Stepwise regression results for maximal bite force in relation to facial dimensions.
(*p<0.05, **p<0.01, †p<0.001)

The results in Table 6.1 show that C-A ($r=.271$, $p<0.05$) and A-M ($r=.215$, $p<0.05$) significantly correlated with maximal bite force (regardless of sex, age or ethnicity). Furthermore, Table 6.2 shows that when C-A ($r^2=.104$, $p<0.05$) and A-M ($r^2=.182$, $p<0.05$) were included in the model, the facial dimensions accounted for a significantly greater amount of variation (18.2% $p<0.01$) in maximal bite force. When all facial dimension variables were included, the model accounted for 19% ($p<0.05$) of maximal bite force variation, regardless of sex, age or ethnicity.

(Correlation with Masseter Muscle Activity)	Pearson's Correlation Coefficient	One-tailed Significance
Upper Facial Height (UFH)	-0.391	0.000†
Lower Facial Height (LFH)	-0.321	0.003**
Condyle to Angle (C-A)	-0.144	0.116
Angle to Menton (A-M)	-0.321	0.003**
Condyle to Angle to Menton (C-A-M)	-0.283	0.008**
Condyle to Condyle (C-C)	-0.007	0.477

Table 6.3: Correlation Coefficient results for masseter muscle activity in relation to facial dimensions.
(*p<0.05, **p<0.01, †p<0.001)

Masseter muscle activity	Model Summary				ANOVA		Standardised coefficients	
	R square	R square Change	F Change	Sig. F Change	F	Sig.	Beta	Sig.
UFH (mm)	0.153	0.153	12.424	0.001**	12.424	0.001**	-0.396	0.001**
+ LFH (mm)	0.158	0.005	0.410	0.524	6.364	0.003**	-0.079	0.603
+ C-A (mm)	0.173	0.015	1.226	0.272	4.665	0.005**	0.148	0.307
+ A-M (mm)	0.174	0.001	0.061	0.806	3.465	0.012*	-0.020	0.908
+ C-A-M (°)	0.177	0.003	0.233	0.631	2.787	0.024*	0.122	0.503
+ C-C (mm)	0.178	0.001	0.097	0.756	2.306	0.045*	0.026	0.838

Table 6.4: Stepwise regression results for masseter muscle activity in relation to facial dimensions.
(*p<0.05, **p<0.01, †p<0.001)

The results in Table 6.3 show that UFH ($r=-.391$, $p<0.001$), LFH ($-.321$, $P<0.01$), A-M ($r=-.321$, $p<0.01$) and C-A-M ($-.283$, $p<0.01$) significantly correlated with masseter muscle activity. Furthermore, Table 6.4 shows that UFH ($r^2=.153$, $p<0.01$) accounted for a significantly greater amount of variation (15.3% $p<0.01$) in masseter muscle activity, which was only improved by 2.5% by the addition of the other facial dimension variables. When all facial dimension variables were included, the model accounted for 17.8% ($p<0.05$) of masseter muscle activity variation, regardless of sex, age or ethnicity.

(Correlation with Temporalis Muscle Activity)	Pearson's Correlation Coefficient	One-tailed Significance
Upper Facial Height (UFH)	-0.039	0.373
Lower Facial Height (LFH)	-0.042	0.364
Condyle to Angle (C-A)	0.146	0.113
Angle to Menton (A-M)	-0.002	0.493
Condyle to Angle to Menton (C-A-M)	0.011	0.465
Condyle to Condyle (C-C)	-0.091	0.225

Table 6.5: Correlation Coefficient results for temporalis muscle activity in relation to facial dimensions.
(*p<0.05, **p<0.01, †p<0.001)

Temporalis muscle activity	Model Summary				ANOVA		Standardised coefficients	
	R square	R square Change	F Change	Sig. F Change	F	Sig.	Beta	Sig.
UFH (mm)	0.002	0.002	0.106	0.746	0.106	0.746	-0.037	0.756
+ LFH (mm)	0.002	0.000	0.030	0.864	0.067	0.935	-0.032	0.845
+ C-A (mm)	0.054	0.052	3.651	0.060	1.263	0.294	0.298	0.057
+ A-M (mm)	0.056	0.002	0.163	0.687	0.977	0.426	0.072	0.696
+ C-A-M (°)	0.069	0.013	0.930	0.339	0.966	0.445	0.181	0.353
+ C-C (mm)	0.076	0.007	0.493	0.485	0.881	0.514	-0.091	0.500

Table 6.6: Stepwise regression results for temporalis muscle activity in relation to facial dimensions.
(*p<0.05, **p<0.01, †p<0.001)

The results in Table 6.5 show that none of the facial dimension variables significantly ($p>0.05$) correlated with temporalis muscle activity. Furthermore, Table 6.6 shows that none of the facial dimension variables significantly explained the variation in temporalis muscle activity. When all facial dimension variables were included, the model accounted for just 7.6% ($p>0.05$) of temporalis muscle activity variation which was not significant.

(Correlation with Ramus BMD)	Pearson's Correlation Coefficient	One-tailed Significance
Upper Facial Height (UFH)	0.018	0.442
Lower Facial Height (LFH)	0.232	0.026*
Condyle to Angle (C-A)	0.047	0.347
Angle to Menton (A-M)	0.189	0.057
Condyle to Angle to Menton (C-A-M)	0.156	0.097
Condyle to Condyle (C-C)	0.173	0.074

Table 6.7: Correlation Coefficient results for ramus BMD in relation to facial dimensions.

(*p<0.05, **p<0.01, †p<0.001)

Ramus BMD	Model Summary				ANOVA		Standardised coefficients	
	R square	R square Change	F Change	Sig. F Change	F	Sig.	Beta	Sig.
UFH (mm)	0.000	0.000	0.022	0.883	0.022	0.883	0.001	0.991
+ LFH (mm)	0.093	0.092	6.911	0.011*	3.467	0.037*	0.434	0.007**
+ C-A (mm)	0.099	0.007	0.511	0.477	2.465	0.070	-0.120	0.424
+ A-M (mm)	0.139	0.039	3.009	0.087	2.657	0.040*	0.326	0.064
+ C-A-M (°)	0.145	0.007	0.517	0.475	2.213	0.063	0.173	0.349
+ C-C (mm)	0.163	0.018	1.355	0.249	2.080	0.068	0.129	0.310

Table 6.8: Stepwise regression results for ramus BMD in relation to facial dimensions.
(*p<0.05, **p<0.01, †p<0.001)

The results in Table 6.7 show that LFH (.232, P<0.05) only, significantly correlated with ramus BMD. Furthermore, Table 6.8 shows that LFH (r^2=.093, p<0.05) accounted for a significantly greater amount of variation (9.3% p<0.05) in ramus BMD, which was significantly (p<0.05) improved to 13.9% with the inclusion of C-A length and A-M length as a whole model. When all facial dimension variables were included, the model accounted for 16.3% (p>0.05) of ramus BMD variation which was not significant.

(Correlation with Mandibular body BMD)	Pearson's Correlation Coefficient	One-tailed Significance
Upper Facial Height (UFH)	0.085	0.242
Lower Facial Height (LFH)	0.130	0.141
Condyle to Angle (C-A)	0.343	0.002**
Angle to Menton (A-M)	0.123	0.154
Condyle to Angle to Menton (C-A-M)	0.066	0.294
Condyle to Condyle (C-C)	-0.138	0.126

Table 6.9: Correlation Coefficient results for mandibular body BMD in relation to facial dimensions.
(*p<0.05, **p<0.01, †p<0.001)

Mandibular body BMD	Model Summary				ANOVA		Standardised coefficients	
	R square	R square Change	F Change	Sig. F Change	F	Sig.	Beta	Sig.
UFH (mm)	0.007	0.007	0.497	0.483	0.497	0.483	0.073	0.543
+ LFH (mm)	0.017	0.010	0.669	0.416	0.582	0.562	0.154	0.352
+ C-A (mm)	0.135	0.118	9.180	0.003**	3.494	0.020*	0.432	0.005**
+ A-M (mm)	0.140	0.005	0.371	0.544	2.689	0.039*	0.131	0.457
+ C-A-M (°)	0.141	0.000	0.029	0.866	2.125	0.073	0.071	0.706
+ C-C (mm)	0.194	0.053	4.238	0.044*	2.566	0.027*	-0.270	0.034*

Table 6.10: Stepwise regression results for mandibular body BMD in relation to facial dimansions.
(*p<0.05, **p<0.01, †p<0.001)

The results in Table 6.9 show that C-A length (r=-.343, p<0.01) significantly correlated with mandibular body BMD. Furthermore, Table 6.10 shows that C-A length (r^2=.135, p<0.01) accounted for a significantly greater amount of variation (13.5% p<0.01) in mandibular body BMD, which was improved to 14% with the inclusion of A-M length as a whole model (p<0.05). The model was then significantly improved (p<0.05) with the inclusion of C-C to explain 19.4% of the variation in mandibular BMD as a whole model (p<0.05), regardless of sex, age or ethnicity.

Discussion

This study examined the effect of facial dimensions obtained through lateral photographs (Figure 6.2) in relation to maximal bite force, maximal masseter and temporalis muscle activity and bone mineral density at the ramus and mandibular body. It specifically sought to explore the relationship between facial dimensions and sample groups, then to explain the variation in key outcome variables (maximal bite force, maximal masseter and temporalis muscle activity and bone mineral density at the ramus and mandibular body) due to differences in facial dimensions.

The ANOVA results highlighted that sample group significantly affected lower facial height, Condyle-Angle-Menton angle, Condyle–Angle length and Condyle-Condyle width. Freitas et al. (2010) reported significant differences between ethnicities in a large cohort of 50 Caucasian Brazilians aged 13.17±1.07yrs and 56 Black Brazilians of

similar age. They reported significant differences between ethnicities in relation to mandibular length (condyle to gnathion), facial convexity and SN-GoGn angle, which is a measure of lower facial height in angular form (33.01±3.98° Caucasians, 30.54±4.42° Blacks, $p<0.003$). The present study didn't measure condyle-gnathion length or facial convexity, but the lower facial height was significantly different between ethnicities in the present findings. However, the present study found African Caribbean males aged 18-25yrs had significantly greater lower facial height than Caucasian males and females aged 18-25yrs, which is in contrast to Freitas et al., (2010), this may be due to the Brazilian hereditary of the Black and Caucasian groups in that study, or to the different techniques used to measure lower facial height. Janson et al. (2011) measured a group of 40 Caucasians aged 13.02yrs and a group of 40 Afro-Caucasians aged 13.02yrs. The Caucasian subjects had significantly greater upper anterior facial height compared to the Afro-Caucasian group, but no significant difference was found for lower anterior facial height. These findings do not fit with the present study, this may indicate the large age difference in facial development between teenagers (as in Janson et al.) and young adulthood (as measured in the present study). Differences in growth patterns and cessation are likely to have affected the results. The discrepancies may also reflect the differences in ethnic cohorts; Janson et al. measured Caucasian and mixed race Afro-Caucasian, whereas the present study compared Caucasian to African Caribbean. In addition, Bacon et al. (1983) reported Cameroon African adult males aged 20-30yrs showed a greater facial convexity, greater lower facial height and a smaller upper facial height compared to their male Caucasian counterparts. This is in accordance with the

present study, which found significantly greater lower facial height in the African Caribbean cohort and non-significantly smaller upper facial height, than the Caucasian cohort. Sexual dimorphism in cephalometric studies has been reported across ethnicities; Gu et al. (2011) showed young adult Caucasian males (mean age 24.1±5.7yrs) exhibited significantly greater lower facial heights than adult Caucasian females, which is reflected in the post hoc results of the present study. As well as sex, age can affect facial dimensions in both males and females, West and McNamara Jr, (1999) reported significant facial dimension changes in males and females from 17yrs to 48yrs in a longitudinal study. Specifically, the study reported an increase in Condyle-Angle length in males, Angle-Menton length increased, upper facial height increased and lower facial height increased with age. Similarly, in the female cohort Condyle-Angle increased, Angle-Menton increased, upper facial height increased and lower facial height increased with age. The present study does not support these findings, the lack of significant difference between young and old indicate that the younger age group were likely to have reached cessation, or were close to cessation, which is unlikely in the 17yrs age group measured by West and McNamara Jr et al., (1999). The sex, age and ethnic differences in facial dimensions presented in previous literature reflect the diversity within the present study cohorts, lower facial height, Condyle-Angle-Menton angle, Condyle–Angle length and Condyle-Condyle width were all significantly affected by the group differences. These findings highlight the successful use of lateral photographs for detecting differences in facial dimensions between groups, instead of using lateral radiographs, which incurs a greater amount of radiation exposure. Moreover, the differences in facial

dimensions may account for the variation in bite force, jaw elevator muscle activity and mandibular BMD across different sexs, age groups and ethnicities.

Bite force

Table 6.1 and 6.2 show that the Condyle-Angle length and the Angle-Menton length significantly correlate with maximal bite force. These two variables significantly explain 18.2% of the variation in maximal bite force. These findings indicate that the length of the mandible, both in height of the ramus and length of the body strongly influence maximal bite force capacity, this may be due to the lever-like qualities of the mandible. Custodio et al. (2011) measured the difference in bite force between groups of men and women (mean age 23.5±4.0yrs) with different facial heights, characterised by 'Brachyfacial', 'Mesofacial' and 'Dolichofacial', which are calculated from key cephalometric measurements including lower facial height and mandibular height. The study reported that Brachyfacial (the shortest anterior facial height) individuals exhibited significantly higher maximal bite force than Mesofacial and Dolichofacial individuals respectively. In a review of bite force studies, Bakke (2006) reported a negative influence of increased vertical facial height, increased mandibular inclination/ increased angle of the ramus, on maximal bite force. The present study did not find a significant influence of facial height on maximal bite force, this may be due to the large variation within the present study. The present study encompassed different cohorts, unlike the previous studies that investigated facial dimensions in one specific sample of people (Proffit et al., 1983; Braun et al.,

1995b). Braun et al. (1995b) reported that a lower mandibular plane angle, which would contribute to a smaller lower facial height, resulted in higher maximal bite force in a cohort of men and women aged 26-41yrs. Similarly, Proffit et al. (1983) reported that long faced individuals had less occlusal force during swallowing, chewing and maximal bite force. Overall, the present study has identified that the length of the ramus and mandibular body contribute significantly to the maximal bite force across the cohorts, this is not in accordance with the previous literature but may be due to the large variation within the sample group.

Muscle Activity

Table 6.3 and 6.4 show that upper facial height, lower facial height, angle-menton length and condyle-angle-menton (mandibular angle) all significantly negatively correlated with masseter muscle activity. Furthermore, upper facial height explained 15.3% of all variation and no other variable improved the model. However, no facial variables significantly explained the variation in temporalis muscle activity. Gomes et al. (2010) reported significantly lower EMG activity during rest in Dolichofacial (long faced) individuals compared to Mesofacial and Brachyfacial (short faced), in the masseter and the temporalis. Furtheremore, these significant differences were observed during maximal biting, in the masseter and the temporalis. These findings may be explained by Van Spronsen et al. (1997), who investigated how the spatial orientation of the jaw muscles related to craniofacial morphology in 30 adult males. The study reported that the condyle-angle-menton (mandibular angle) and the

posterior facial height (influenced by the height of the ramus) largely explained the variation in temporalis and masseter muscle orientation. The orientation of the jaw elevator muscles, influenced by craniofacial morphology, may have subsequently affected the muscular activity during contraction. The previous findings identify a negative relationship between facial height and EMG activity, specficically, individuals with short faces exhibit higher masseter and temporalis EMG activity during biting. The present study also found a significant negative relationship between facial height and masseter muscle activity, but this was not replicated in the temporalis muscle. Furthermore, Raadsheer et al. (1996) measured facial dimensions and muscle thickness in a large cohrot aged 7-22yrs, the study reported similar findings to Gomes et al. (2010) concerning a negative relationship between masseter muscle thickness and anterior facial height, as well as mandibular length. However, the largest portion of variation was attributed to age, stature and weight, which is expected due to the large variations in size between males and females aged 7yrs and 22yrs. The present study also attrubutes variations in bite force, muscle activity and BMD to the differences in sex, age and ethnicity as discussed in Chapters 3-5, however the focus of this Chapter was to discover the extent to which facial dimensions influence variation in the outcome variables, regardless of sample groups. Some studies (Gomes et al., 2010; Custodio et al., 2011) have shown facial height to have a significant effect on masseter muscle activity, which is in accordance with the present study findings.

Bone Mineral Density

Table 6.7 and 6.8 identified a significant correlation between lower facial height and ramus BMD, which also explained 9.3% of variation in ramus BMD. However, the addition of condyle-angle length (ramus length) and angle-menton length (mandibular length) explained 13.9% of the variation in ramus BMD. Table 6.9 and 6.10 show that the condyle-angle length (ramus height) significantly correlated with mandibular BMD and explained 13.5% of the BMD variation. There are few research studies that report BMD of the craniofacial skeleton, with which to compare the present study findings. However, Algaidi and Elsaed (2012) examined the differences in facial dimensions between healthy (mean age 56 years) and osteoporotic (mean age 59 years) Egyptian men and women. Facial measurements were examined by hand using a vernier sliding caliper, instead of using cephalometric measurements from lateral radiographs, which is a different technique to the present study and may explain some differences in findings. The results showed significantly greater lower facial height in osteoporotic men and in osteoporotic women. Furthermore, the gonion-menton length (mandibular body length) was significantly larger in female osteoporotic patients than healthy controls, but not in men. The study also reported that upper facial length was significantly higher in osteoporotic women compared to controls, but osteoporotic males exhibited smaller upper facial height than healthy controls. These findings show a negative relationship between lower facial height and BMD (decreased BMD linked to increased lower facial height) as well as a negative relationship between mandibular length and upper facial height

with BMD in females (decreased BMD linked to increased upper facial height and mandibular length). The study offered little explanation for these differences, and the findings of the present study are in disagreement. This study found a positive relationship between mandibular and ramus BMD and the mandibular lengths, in healthy populations. The positive relationship between mandibular lengths and BMD in the present study reflect the mandible as both a lever and a link system (Gingerich, 1979). The muscles provide an applied force, which results in a resultant bite force and a reaction force at the mandibular joints. In a link system the applied muscle force equals the resultant bite force, if only the muscles aligned with the tooth row (middle and posterior temporalis) are utilised, but if the other elevator muscles (masseter, anterior temporalis and medial pterygoid) are utilised, the lever system produces a reaction force at the mandibular joints as well. During mastication in humans, it is possible to switch between lever, link and both (Gingerich, 1979). The applied force is transferred to the bone at the muscular attachment sites, the bite force is generated at the tooth row, which transfers force to the bone below the teeth and the resultant force is concentrated at the mandibular joints. These areas of the mandible therefore require bone material properties that can withstand compression, tension, shear and torsional loads (Van Eijden, 2000), which will have a positive effect on the bone. The positive link between BMD and facial dimensions found in the present study may be due to the muscle-bone relationship, specifically the influence of muscle pull on the osteogenic response of bone tissue.

Conclusion

This study aimed to investigate how facial dimensions influenced bite force, jaw elevator muscle activity and BMD in the mandible, regardless of sex, age and ethnicity. The findings show that the facial dimensions account for some of the variation in outcome variables; C-A length and A-M length account for 18.2% of bite force variation. Furthermore, UFH accounts for 15.3% of all masseter muscle activity variation, LFH accounts for 9.3% of ramus BMD, which was increased to 13.9% with the inclusion of A-M length and C-A length, whilst A-M length accounted for 14% of the variation in mandibular BMD. It is pertinent to acknowledge that the overwhelming effect on all of the outcome variables is likely to be caused by age and/or ethnicity. However, the influence of the facial height measurements and the length of the ramus and mandibular body are most likely due to the biomechanical structure of the mandible, for example, the significantly positive influence of mandibular length on ramus and mandibular body BMD. These findings may reflect the muscle pull and force distribution patterns experienced during mastication, which occurs across all sample groups.

Chapter 7: Discussion

The aims of the present studies were to investigate the relationship between bite force, jaw elevator muscle activity and mandibular bone mineral density. In addition, the study examined the effects of sex, age, ethnicity and facial dimensions on the muscle – bone - bite force relationship. Research has documented the relationship between force and muscle activity in relation to the whole body (Disselhorst-Klug et al., 2009), particularly between muscle pull and BMD at the hip (Ahedi et al., 2014) and bone mineral content at the arm (Ireland et al., 2014). However, few studies have reported both bite force and muscle activity or BMD in the craniofacial skeleton. This study aimed to connect all three variables within the craniofacial skeleton, in particular the bite force, jaw elevator muscle activity and mandibular BMD. This appears to be the first study to combine bite force, muscle activity and BMD of the mandible as a set of outcome variables. The following section will discuss the findings of this study and previous studies, emphasising the differences in bite force, muscle activity and BMD as well as the effect of facial dimensions on each of these variables.

7.1 Bite Force

Sex

The present study found no significant difference in bite force between young adult males and females. A number of studies found significant differences between young

adult males and females with regard to bite force (Ferrario et al., 2004; Koc et al., 2010; Palinkas et al., 2010). Ferrario, et al. (2004) reported significantly higher bite forces in males than females, at all tooth positions from incisors to 2nd molars. Palinkas et al. (2010) reported a significant effect of sex on maximal molar bite force in young people aged 13-20yrs and young adults aged 21-40yrs, which suggests that sexual dimorphism is developed during mid to late puberty and extends into adulthood. However, the present study findings concur with Lepley et al. (2011) who reported no significant differences between young adult males (*n*=15) and females (*n*=15) aged between 22 and 32 years old, on both premolar and molar dentition, using a custom made bite force device. Other studies that have yielded non-significant differences between sexs include Motegi et al. (2009) who used an older Japanese cohort (aged 60-80+yrs) and Paphangkorakit and Osborn (1997) who measured young adults aged 28-36yrs but only used a small cohort (8 males and 2 females). Further studies include, Thompson et al. (2001) who measured a small cohort (7 males and 7 females) of young adults aged 22-35yrs but pooled the results and Caloss et al. (2011) who measured bite force in a small cohort (7 males, 10 females) of denture wearers but pooled the results. Bakke et al. (1990), suggested that sample groups as low as 8-14 people, used in bite force studies may be insufficient for detecting the sex difference from craniofacial morphology or occlusal variability. It is apparent that the sample size used in the present study could have hindered the significant differences between sexs in the young adult group. It appears that young adults with sound dentition require larger sample groups because variability due to other factors such as facial morphology and masticatory

muscle thickness have been found to account for up to 58% of variance in bite force (Raadsheer et al., 1999). Furthermore, in the present study, the standard deviation from the mean bite force in the young adult population, particularly Caucasian males (±142.6N), is noticeably higher than the older population (±97.1N pooled), which indicates a greater amount of variability.

Ethnicity

In relation to ethnicity, Caucasian adult males showed lower mean bite force than African Caribbean adult males, but this difference was also not significant. The uneven sample sizes used to measure differences in ethnicity could have contributed to the non-significant findings, which showed a large difference between Caucasian and African Caribbean males mean bite force. Differences in bite force between ethnicities have been reported in previous literature between Indigenous and White Brazilian males and females (Regalo et al., 2008) and young adult Japanese and Danish females (Shinogaya et al., 2001). This indicates that ethnicity differences in bite force is a young area of research, with most papers focussing on one specific ethnicity rather than exploring the differences in bite force between two or more ethnicities. A limitation of the study is the size of the sample groups, the Caucasian cohorts each contained 15 males or females and the African Caribbean group contained 12 males. The present study began to recruit African Caribbean young adult females and over 50 year olds, but was limited by time and location and could not fulfil all sample groups. Participants were recruited by word of mouth,

newspaper articles and local exercise groups for mature adults. As the data collection only required one visit, there were no 'drop outs' but 3 participants had to be eliminated due to poor bite force. Despite the non-significant results, the present findings do highlight a large mean difference (98N) between Caucasian and African Caribbean males. Furthermore, a comparison of young adult females and older African Caribbean males and females with Caucasian counterparts would add to the understanding of how bite force, muscle activity and BMD are affected by age, across ethnicities. To the author's knowledge, no papers have addressed the differences in bite force between young adult males of African Caribbean and Caucasian ethnicity with muscle activity and bone mineral density. This is a novel element of this research study.

Age

The effect of age on bite force was found to be significantly negative in a female cohort in the present study, but not in the male cohort. The >50yrs males exhibited a 36.6N decrease and the >50yrs females experienced a 104.5N decrease in bite force compared to their <25yrs counterparts. These findings indicate a reduction in bite force with increasing age, which is concurrent with studies that describe a reduction in molar bite force (Palinkas et al., 2010) in adults, as age increases. In addition, these results are fairly concurrent with Palinkas et al. (2010) who reported an 82N maximal bite force reduction in males and 118N maximal bite force reduction in females, between a 13-20yrs age group and a 41- 60yrs age group. Although the

>50yrs male cohort was not significantly lower (which may be due to slower loss of muscle activity and bite force in males with ageing) the findings do indicate a similar trend in the data. The reduction in maximal bite force due to poor or false dentition, may be reflected in the present cohort of >50s, which consisted of 63% mixed dentition, of which 11% wore dentures.

Facial Dimensions

The present study found the Condyle-Angle length and Angle-Menton length significantly correlated with maximal bite force and these two variables significantly accounted for 18.2% of the variation in maximal bite force across all sample groups. These findings indicate that the shape of the mandible, both in height of the ramus and length of the mandibular body, strongly influence maximal bite force capacity. Custodio et al. (2011) reported that Brachyfacial (short anterior facial height) individuals exhibited significantly higher maximal bite force than Mesofacial (average facial height) and Dolichofacial (long facial height) individuals respectively. Similarly, Proffit et al. (1983) reported that long faced individuals had less occlusal force during swallowing, chewing and maximal bite force. Conversely, the present study did not find a significant influence of facial height on maximal bite force, neither measure accounted for a significant portion of the variation across cohorts. This may have been influenced by the measurement technique that utilised lateral photographs rather than radiographs. Overall, the present study has identified that the length of the ramus and length of the mandibular body contribute significantly to the maximal

bite force across the cohorts, this supports the biomechanical theory of the mandible as a lever, but the role of the jaw elevator muscles in bite force has not been fully explained in this study.

7.2 Bite Force and Muscle activity

Sex

The present study results found a significant correlation between bite force and masseter muscle activity in young adult males, as well as a significant correlation between masseter and temporalis muscle activity in young adult females. These findings are concurrent with Ferrario et al. (2004) who reported bite force to have a linear relationship with masseter and anterior temporalis muscle activity, in a cohort of young (aged 20-29yrs) healthy volunteers. Other studies have reported mixed results in relation to bite force and muscle activity between sexs. Lindauer et al. (1993) found no significant differences in EMG-force slopes in young adult males and females, whilst Fogle and Glaros (1995) reported no significant differences in incisal bite force and muscle activity at the masseter and temporalis between sexs, in a cohort aged 18-45yrs. In contrast, Ferrario et al. (1993) found significantly higher masseter muscle activity in males than females during maximal clenching, in a large cohort males and females aged 20-27yrs. The differences between male and female muscle activity were evident, but were not significant in the present study. This may be linked to the large variation in normalised (%) muscle activity, which is large in

comparison to other measurements and may be rectified by increasing the sample sizes.

Ethnicity

In the present study, a significant correlation existed between maximal bite force and maximal masseter muscle activity, as well as between maximal masseter muscle activity and maximal temporalis muscle activity, in the African Caribbean cohort. The strong correlations between muscle activity and bite force within the African Caribbean cohort, were reflected in the male Caucasian cohort, but the link between masseter and temporalis muscle activity was not. Paphangkorakit and Osborn (1997) reported lower anterior temporalis muscle activity during biting maybe due to the fact that the temporalis only reaches its peak contraction at wide mouth opening heights, such as when biting into an apple. The significant correlation between masseter and temporalis muscle activity in the African Caribbean cohort only, may reflect the ethnic differences in craniofacial morphology and indicate their influence on muscle activation.

Age

In the present study, there was no significant relationship between bite force and muscle activity in the >50yrs cohorts, but a significant correlation between masseter and temporalis muscle was found in both the >50yrs males and females. Previous

studies have reported the relationship between muscle activity and bite force in edentulous or older cohorts (Van Der Bilt et al., 2008; Caloss et al., 2011). Caloss et al. (2011) reported EMG activity and bite force did not follow the same pattern in denture wearers, when the muscle activity was significantly different, the bite force was not. However, in a study of 19-69 year old dentate men and women, Van Der Bilt et al. (2008) reported that the bilateral and unilateral bite forces correlated to bilateral and unilateral muscle activity, as well as a significant negative correlation between bite force, and the corresponding muscle activity with advancing age (Van Der Bilt et al., 2008). The present findings are similar to Van Der Bilt et al. (2008) with regards to the negative effect of age on bite force and muscle activity in the female cohort, however, the present study does not reflect the correlation between bite force and muscle activity reported by Van Der Bilt et al. (2008). The age related changes in facial morphology may contribute to the reduction in bite force in an older cohort. Furthermore, Galo et al. (2007) reported a lower mean muscle activity in the elderly compared to the young, when chewing hard foods but no difference in consistency at lower levels, which suggests the elderly remain efficient at low bite force levels. These findings are concurrent with the present study, which found no significant differences in muscle activity at 25%, 50% and 75% bite force levels between age groups but did find a significant reduction in >50yrs females during maximal bite force. These findings suggest that the masticatory system continues to function at a submaximal level throughout ageing but maximal muscle contractions or bite forces become increasingly reduced, particularly in post-menopausal women.

7.3 Muscle Activity

Sex

The present study found no significant difference in muscle activity between young adult Caucasian males and females. These findings are concurrent with Ferrario et al. (2000), who conducted a facial EMG study on a similar aged cohort that also measured muscle activity from the masseter and anterior temporalis. They found no sex differences in muscle activity, which is concurrent to the present study findings. Furthermore, Lindauer et al. (1993) reported EMG-force slopes for 8 young adult males and 8 females during muscle contraction at varying mouth opening heights, the study found no significant differences in sex with regard to muscle activity. The female cohort in the present study exhibited a correlation between masseter muscle activity and temporalis muscle activity, but the male cohort did not demonstrate a similar significant correlation. Lindauer et al., reported the anterior temporalis exhibited a similar pattern of muscle activity as the masseter during maximal biting, but their findings were not significant. This is directly reflected in the present study results and the sample size may not have been large enough to detect a significant difference in muscle activity.

Age

This study did find a significant difference in jaw elevator muscle activity between <25yrs Caucasian adults and >50yrs Caucasian adults. Facial EMG studies tend to

report muscle activity in differing sample groups such as variations in craniofacial shape (Suvinen and Kemppainen, 2007), long term migraines (Burnett et al., 2000) and osteoporosis of the jaw (Siéssere et al., 2009). The findings of such studies can be difficult to compare to the present study, which was conducted on healthy individuals that were not selected based on their facial dimensions or dentition. In the present study, the >50yrs cohort comprised of 37% full dentition and 63% mixed dentition, of which 11% wore dentures. Caloss et al. (2011) found that occlusal instability in denture wearers during maximal biting, resulted in lower facial muscle activity compared to stable bilateral biting. The reduction in maximal muscle activity due to instability may be reflected in the present >50yrs female cohort, who exhibited a significantly lower muscle activity than the <25yrs female group. A similar reduction was not found in the male cohort. This may reflect the skeletal changes in post-menopausal women compared to the slow changes observed in men with ageing. Despite the loss of maximal muscle activity associated with age, there was no significant difference in muscle activity at 25%, 50% and 75% bite force levels between age groups. These findings suggest that the jaw elevator muscles continue to function simultaneously at submaximal bite forces but maximal muscle contractions in the masseter and anterior temporalis become increasingly reduced.

Ethnicity

The present study found no significant effect of ethnicity on muscle activity in either the masseter or temporalis, during sub-maximal and maximal contractions. Caucasian males exhibited a non-significant correlation between masseter and temporalis activity, whereas African Caribbean males exhibited a significant relationship between the elevator muscles. Differences in jaw muscle force are confounded by the complex differences in jaw size and facial morphology. This may also explain the difference in muscle activity correlation between the two cohorts, as African Caribbean skull morphology is different to Caucasian (Connor and Moshiri, 1985; Flynn et al., 1989) and may result in different lengths of muscle between attachment sites, different angles of muscle in relation to the craniofacial structure and different patterns of activation during movement. Moreover, the present study only measured activity from the masseter and anterior temporalis, which may under represent the contribution provided by other masticatory muscles such as the medial and lateral pterygoids and the digastric. Future research could explore more of the jaw elevator muscles during bite force and mastication, to ascertain the contribution that each makes during maximal bite force, particularly across age groups and ethnicities.

Facial Dimensions

Upper facial height, lower facial height, angle-menton length and condyle-angle-menton (mandibular angle) all significantly correlated with masseter muscle activity in the present study. Furthermore, upper facial height explained 15.3% of all variation. However, no facial variables significantly explained the variation in temporalis muscle activity. Van Spronsen et al. (1997) reported that the condyle-angle-menton (mandibular angle) and the posterior facial height (influenced by the height of the ramus) largely explained the variation in temporalis and masseter muscle orientation. In terms of muscle activity, the difference in muscle position is likely to alter the contraction pattern of each elevator muscle. These findings are reflected in the present study, which also linked mandibular angle to the masseter muscle activity. Similarly, Gomes et al. (2010) reported significantly lower EMG activity during maximal biting in Dolichofacial (long faced) individuals compared to Mesofacial (average) and Brachyfacial (short faced), in the masseter and the temporalis. These findings identify a negative relationship between facial height and EMG activity, specifically, individuals with short faces exhibit higher masseter and temporalis EMG activity during biting. The present study also found a significant negative relationship between facial height and masseter muscle activity, but this was not replicated in the temporalis muscle. The lack of findings in the temporalis muscle activity across all participants, may indicate a low sensitivity in the temporalis to changes in mouth opening or bite force which relates to Paphangkorakit and Osborn (1997). Within the present study, the jaw dimensions were calculated from

photographs, using a computer software package (ImageJ) that calculates angles and lengths when calibrated. This approach is uncommon in facial bone research, as cephalometric measurements taken from lateral radiographs are widely favoured. The measurements calculated from photographs are unlikely to be comparable to cephalometric data as the techniques used are very different. However, photographs are a non-invasive technique that poses no additional harm to participants, the use of radiographs expose participants to (sometimes unnecessary) radiation. Future research may aim to ratify this technique in research, providing a reliable, structured protocol and may utilise the already common clinical photographic assessment of facial dimensions.

7.4 Muscle Activity and BMD

The present study found no correlations between muscle activity and mandibular BMD in any of the individual cohorts. The only group exhibiting an average positive correlation were the African Caribbean group, between the masseter and the ramus, but this was not significant. An effect of muscle activity on BMD was expected in the study based on Wolffs Law, the Mechanostat theory, which detail the relationship between muscle strain and BMD. The frequency and magnitude of the loads exerted on bone define the level of response. Forces applied to the craniofacial skeleton tend to be low, except during trauma. These low forces may be produced by muscle contraction during movement of the head or jaw (Kiliaridis, et al., 1995; Van Spronsen et al., 1997). The present study aimed to identify a link between muscle

activity in the master and anterior temporalis and BMD of the mandible on the corresponding muscle attachment sites on the mandible. The findings corroborate a non-significant relationship between maximal muscle activity and BMD of the mandible, however this may be affected by a number of variables such as sample size and occlusal factors such as dentition. No previous studies have related jaw elevator muscle activity to BMD of the facial bones, in particular the mandible. This may be due to problems with the experimental design such as sample size, or it may indicate that BMD is less susceptible to change due to muscle pull in the jaw or that other bone parameters such as cortical bone thickness are more closely related to muscle pull than BMD, for example cortical bone thickness or masseter muscle thickness. Masseter muscle thickness has been linked to differences in mandibular alveolar bone mass (Jonasson and Kiliaridis, 2004) and to differences in facial morphology (Kiliaridis and Kälebo, 1991), and could serve as an additional component to the muscle-bone-bite force relationship described in this study. It is possible that muscle thickness and certainly cortical bone thickness could give a more detailed insight into the loading history of the jaw and jaw muscles. It would be ideal to couple these variables with the existing mandibular BMD and jaw elevator muscle activity, not only to compare the relationships between the measurements, but also to provide additional understanding to the craniofacial mechanics.

7.5 Bone Mineral Density

Young adult Caucasian males exhibited consistently higher BMD values than Caucasian females except for the lumbar spine. However, these findings were non-significant at all skeletal sites. Previous studies that have focussed on sex differences in bone mineral density, have shown that BMD differences in young adults can change or become non-significant when corrected for anatomical size (Henry and Eastell, 2000; Peacock et al., 2009). Henry and Eastell (2000) found men (mean age 26.3 years) had significantly greater bone area at the total body, lumbar spine and femoral neck as well as significantly higher BMD at corresponding sites, than females. Nevertheless, when BMD was corrected for bone area, males had lower BMD at the lumbar spine and similar BMD at the femoral neck. This change in BMD difference may be explained by the planar nature of DXA, which measures the two dimensional areal BMD rather than volumetric BMD, and may therefore under represent the density of the whole bone at skeletal sites (Riggs et al., 2004; Peacock et al., 2009). The present study modified the analysis technique for femoral neck BMD, so that the area of bone used for analysis was the same for every participant, regardless of bone size. This process may have indirectly corrected for anatomical size differences between the two sample groups, which may explain the non-significant differences found between males and females, and is concurrent with the previously mentioned literature. The <25yrs female sample group exhibited a significant correlation between mandibular BMD and the lumbar spine as well as an average correlation between femoral neck and the lumbar spine, which was not

significant. The male cohort showed no correlation between any skeletal sites. The present study's use of DXA for determining the effect of muscle activity and bite force on BMD in healthy subjects may be considered a limitation. Although DXA is widely used for determining bone health through BMD values, particularly in relation to metabolic bone disease such as osteoporosis, other measurements of bone may give more indication of the loading history. For example, quantitative computer tomography (qCT) can indicate volumetric BMD and in particular cortical density, which may provide a better understanding of the structural and material changes of bone due to the forces applied. DXA is ideal for research studies because it uses a very small amount of radiation compared to CT or a conventional X-ray, but in order to find out more specific information in future, a compromise on radiation levels will need to be made. The research field would benefit from more studies that investigate the BMD across skeletal sites, including the craniofacial structure, in healthy individuals as a baseline data comparison for more complex groups. This information would be of particular use in facial sports injury, in a young adult cohort who have the highest participation rates in high impact, high risk sports.

Ethnicity

In terms of ethnicity, young adult Caucasian males exhibited consistently lower BMD values than African Caribbean males. These findings were significant at the mandibular ramus, femoral neck and lumbar spine ($p<0.05$). These findings are similar to Wagner and Heyward (2000) who reported definitive differences between

black and white populations in total body bone mass, bone mineral density and bone mineral content, which were consistently greater in black populations. Additional studies have found black male populations to have consistently higher BMD at skeletal sites than Caucasian males (Nelson et al., 1995; Ettinger et al., 1997; Wang et al., 1997; Looker et al., 2009). The present study found African Caribbean males exhibited a 22% greater BMD at the femoral neck, and a 21% greater BMD in the lumbar spine, than Caucasian males. Ettinger et al. (1997) reported young black men exhibited, on average, a 12% greater BMD at the lumbar spine and a 20% greater BMD at the femoral neck compared to Caucasian. In terms of femoral neck and lumbar spine BMD, the present study findings are concurrent with previous findings but there were no values for mandibular BMD in the previously mentioned studies, which measured the same ethnicities and similar age groups. A study by Ong and Stevenson (1999) found Australian males and females of Asian descent had 20% higher BMD at the mandibular angle than those of Caucasian decent, using measurements from radiographs. Although the ethnic groups are different to the present study, it does indicate a strong significant difference in mandibular angle BMD between ethnicities, which is concurrent with the present study. A significant correlation between the mandibular body and the ramus BMD in the African Caribbean cohort, may indicate a more uniform distribution of BMD throughout the mandible, which may reflect the different facial morphology or loading patterns in the African Caribbean craniofacial skeleton. The use of DXA to examine BMD of the mandible in a young healthy population has not been replicated in previous research, except for a study by Li et al. (2011) that focussed on the correlation

between mandibular angle, mandibular symphysis and lumbar vertebrae BMD in a large Chinese cohort.

Age

In a study of healthy, edentulous females aged 44-79yrs (mean 65yrs) Horner et al. (1996) reported significant correlations between the mandibular ramus and mandibular body, as well as the mandibular body and the mandibular symphysis. These findings indicate a relationship between facial bone BMD by DXA in an older, edentulous population which has not, to the author's knowledge, been replicated in a young and/or multiracial cohort. The <25yrs cohort exhibited greater BMD values than the >50yrs cohort, except for at the mandibular ramus. These findings were significant at the mandibular body and femoral neck in the >50yrs females only. Studies that show a reduction in BMD due to ageing are commonplace, particularly with such a defined age gap between the two cohorts, as demonstrated in the present study. Devlin and Horner (2007) reported a reduction in mandibular body and ramus BMD with increasing age in a cohort of 72 females, average age 62 years. The values presented in the paper were similar to those found in the present study for the mean BMD of the mandibular body, which indicates that the effect of ageing on BMD in the mandible is similar to other skeletal sites, particularly in post-menopausal women. However, the results from the present study show an increase in ramus BMD in the over 50s cohort compared to the under 25s. This is an unexpected finding, which may be explained by the structural changes in the

mandible with age or may be specific to the sample of males and females tested in the present study. Drage et al. (2007) found the ramus BMD strongly correlated with the BMD at the hip and lumbar spine in edentulous participants and both the ramus and hip exhibited a negative relationship with increasing age. This was not reflected in the present findings, but a correlation of lumbar spine with mandibular body was observed in the >50yrs males cohort. This may be due to the variation in loading patterns in the mandible and lumbar spine in relation to the femoral neck. These findings are comparative to Drage et al., (2007) who also found correlations between facial BMD and the lumbar spine, in a cohort of men and women aged 67.1±12.6yrs. These studies were conducted using participants from the same age range as the present study, and they indicate a strong correlation between BMD at facial and loaded skeletal sites in an ageing population. The findings of the present study relate to a healthy ageing population, the majority of which had permanent dentition, were physically active and free from metabolic bone diseases. The present study used an adapted analysis technique for deriving BMD values from DXA, despite the different approach, the present study reported similar values for mandibular, lumbar spine and femoral neck BMD to previous studies.

Facial Dimensions

In the present study, lower facial height significantly correlated with ramus BMD, which explained 9.3% of variation in ramus BMD. However, with the addition of condyle-angle length (ramus length) and angle-menton length (mandibular length)

13.9% of the variation in ramus BMD could be explained. In addition, the condyle-angle length (ramus height) correlated significantly with mandibular BMD. To the authors knowledge, only one research study has previously compared BMD of the facial bones to facial dimensions, using cephalometric analysis. Algaidi and Elsaed (2012) reported significantly greater lower facial height in osteoporotic men compared to healthy men and in osteoporotic women compared to healthy women. Furthermore, the gonion-menton length (mandibular body length) was significantly larger in female osteoporotic patients than healthy controls, but not in men. The study also reported that upper facial length was significantly higher in osteoporotic women compared to controls, but osteoporotic males exhibited smaller upper facial height than healthy controls. Little explanation was offered for these differences, and the findings of the present study are in direct disagreement. The present study found a positive relationship between mandibular BMD and ramus and mandibular lengths, in healthy populations. These findings reflect the mandible as a both a lever and a link system (Gingerich, 1979), which encapsulates the notion of the force-muscle-bone relationship. The muscular attachment sites transfer the muscle force to the bone, the tooth row transfers bite force to the mandibular body and the resultant force is concentrated at the mandibular joints. The research field would benefit from further exploration of this relationship, in order to better understand the functionality of the masticatory system.

7.6 BMD and Bite Force

Sex and Ethnicity

The present study found no significant correlations between bite force and mandibular BMD in any of the young adult cohorts. However, the African Caribbean group exhibited a positive correlation ($r=0.57$, $p>0.05$) between bite force and BMD at the ramus as well as considerably higher mean bite force values than their Caucasian counterparts, but this difference was also non-significant ($F(1,25)2.72$, $p>0.05$). These correlations, although not significant, were expected within this study; they indicate a relationship between greater bite force and increased site specific BMD at the mandible. It is possible that the smaller sample sizes confounded the significance of these correlations and inter-ethnic differences. Differences in facial morphology (Farkas et al., 2005) and size of dentition (Merz et al., 1991) between Caucasian and black groups may influence the aptitude of the mandible as a lever, facial BMD and may indicate a prevalence for stronger bite forces in one particular population. However, there are no previous bite force studies that investigate African Caribbean groups to confirm the differences found in the present study. Furthermore, there are no studies on young adults, that compare bite force and BMD in the craniofacial skeleton by DXA, to compare these findings to.

Age

Within the present study the only cohort to exhibit a connection between bite force and BMD was the >50yrs group. Bite force significantly correlated with BMD of the

ramus in >50yrs males and mandibular body in >50yrs females. Reduced bite force has been linked to extreme reductions in bone mineral density. Siéssere et al. (2009) showed osteoporotic women exhibited significantly lower bite force than healthy controls. Previous studies that have measured craniofacial BMD in older sample groups have not measure bite force in relation to BMD (Pluskiewicz et al., 2000; Drozdzowska and Pluskiewicz, 2002; Horner et al., 2002; Lindh et al., 2004; Devlin and Horner, 2007; Drage et al., 2007), this indicates a gap in the research field. The relationship between bite force and BMD in an older population may well simply reflect the process of ageing on the aptitude and integrity of the masticatory system, or it might highlight an underlying relationship between force and BMD in the facial skeleton. The present study links bite force and mandibular BMD in a single healthy, ageing population, which to the author's knowledge, has not been reported in previous studies.

The Overall Relationship

There is evidence from the present study to suggest that the link between bite force, muscle activity and BMD may be more evident in the African Caribbean group, however the size of the sample groups likely hindered the discovery of significant results. Due to the large number of facial dimensions and the complex nature of the jaw as a biomechanical link and lever, many variables may confound the discovery of significant results. Nevertheless, these facial variables contribute to our understanding of the intricate relationship between bite force, jaw elevator muscle

activity and BMD of the mandible. There are stronger relationships between bite force, muscle activity and BMD in the >50yrs group, but it is possible that this is due to the overall effect of ageing on force production, dentition, muscle strength and BMD is masking the lack of relationship between the three main outcome variables. Conversely, it may be that the underlying relationship between force, muscle activity and BMD are accentuated during ageing. Further exploration of the relationship between these three components is necessary to discover whether they can be utilised to improve the strength of the jaw, and to what extent they can be utilised in different groups of people. An expansion of the sample sizes, groups (particular expansion into more ethnic groups) and additional measurement techniques are advisable.

Chapter 8: Conclusion

The present study aimed to identify a relationship between force, muscle activity and bone mineral density in the craniofacial skeleton, specifically bite force, jaw elevator muscle activity and mandibular BMD. The present study did not accomplished this aim in its entirety; the three-way relationship between bite force, jaw elevator muscle activity and mandibular BMD was not observed in any one sample group, but significant relationships between these variables were observed, some provide new insights into our understanding of the mandible as a lever. This lack of significant findings may be due to inadequate sample sizes or variations in measurement techniques compared to other research studies.

This study developed a new bite force device based on existing technologies and used an computer system that synchronised bite force and muscle activity, which has not been reported in previous bite force or facial muscle activity research. The present study also built upon previous research that had developed DXA scanning techniques for measuring maxilla and mandibular BMD. A different analysis technique was developed for use in this study and the positioning of participants during facial scans was adapted from previous literature.

This study did identify correlations between bite force and masseter muscle activity in a cohort of young adult Caucasians, which highlights the functionality of the masticatory system in young adults and may be of benefit as a baseline with which to compare other sample groups. Furthermore, the findings highlighted a gross reduction in BMD in post-menopausal women compared to young adult women,

which was also significant in the mandibular body. In a healthy population, free from metabolic bone disease, both men and women may experience significant decreases in mandibular BMD, which could have detrimental effects in the event of a fall or impact to the jaw. This study also compared bite force, muscle activity and mandibular BMD in an African Caribbean cohort, which has not been reported in previous literature. The study identified that the ethnic differences in BMD extend to the mandible, which is reflected in the greater occurrence of facial injury in sport in Caucasian males compared to African Caribbean males. The findings of the present study that relate to young adults may inform the practices of facial protection in sport and in relation to older men and women participating in sport, protective equipment is of the utmost importance, particularly that which is custom made and of high quality.

Appendix

A

Can Masticatory Electromyography be Normalised to Submaximal Bite Force?

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Running Head: **Normalise EMG to Submaximal Bite Force**

Article category: **Original research article**

Abstract

The combination of bite force and facial electromyography (EMG) provides an insight into the performance of the stomatognathic system, especially in relation to dynamic movement tasks. Literature has extensively investigated possible methods for normalising EMG data encapsulating many different approaches. However, bite force literature trends towards normalising EMG to a maximal voluntary contraction (MVC), which could be difficult for ageing populations or those with poor dental health or limiting conditions such as temporomandibular disorder. The objectives of this study were to (i) determine whether jaw-closing muscle activity is linearly correlated to incremental sub-maximal and maximal bite force levels, and (ii) assess whether normalising maximal and submaximal muscle activity to that produced when performing a low submaximal bite force (20N) improves repeatability of EMG values. Thirty healthy adults (15 male, 15 female; mean age 21±1.2 years) had bite force measurements obtained using a custom-made button-style compression load cell. Masseter and anterior temporalis muscle activities were collected bilaterally using surface EMG sensors whilst participants performed maximal biting, and three levels of submaximal biting. Furthermore, a small group (n=4 females) were re-tested for reliability purposes. Coefficients of variation and intraclass correlation coefficients showed markedly improved reliability when EMG data were normalised compared to non-normalised. This study shows that facial EMG may be successfully normalised to a very low bite force. This may open possibilities for comparisons between at-risk sample groups that may otherwise find it difficult to produce maximal bite force values.

Keywords: Normalisation, Masticatory muscles, Masseter muscle, Temporal muscle, Muscle activity, Bite force.

B

The relationship between sex and age on mandibular bone mineral density and key anatomical sites as a potential key indicator for the use of facial sports protection.

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Objectives

Injuries to the mandible have been shown to be one of the highest orofacial fracture rates in sport (1). Bone mineral density (BMD) and structure are integral to bone strength (2). Thus the relationship between whole body, facial BMD and lifestyle differences could be indicative factors for influencing oral/facial protection in sport, especially for those age- or sex-related sample groups whom maybe at greater risk of injury.

Method

Adult participants (n=30 male, 30 female) were divided equally into two groups; <25 years (mean age 21±1.85) and >50 years (mean age 62±7.88). BMD values were obtained using a Discovery QDR dual energy X-ray absorptiometry (DXA) scanner (Hologic Inc, USA). Specifically, lumbar spine BMD was calculated as a mean of L1-4 (g/cm^2) and femoral neck BMD calculated by using a $2.5cm^2$ rectangular window (along the femoral neck width). Facial BMD was measured bilaterally using the forearm scan software and calculated using 4 x $0.22cm^2$ analysis windows, at both the angle of the ramus and lateral edge of the mandible.

Results

Multivariate statistical analysis was conducted on separate sex and age groups. No significant differences were detected in any of the BMD sites between 'sex' and 'sex and age' combined. However, there was a significant difference between age groups, irrespective of sex, in BMD across combined sites ($p<0.01$).

Conclusion

Sex did not have an effect on BMD at the hip, lumbar spine, ramus and mandible as a collective but age did show an effect on BMD at facial and load bearing sites. Thus, with use of DXA increasing amongst sports participants it could be a useful screening tool for practitioners to highlight the importance of facial protection, especially in older athletes and those with lower BMD values.

C

Facial Bone Mineral Density: A potential indicator for at risk injury groups.

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Sports related facial injuries are most prevalent amongst young men aged 16-30 yrs, with male to female injury ratios as high as 19:1. The most common site of injury is the mandible followed by the mid-face. The severity of injury can have a detrimental effect on health and return to sport. Differences in facial structure and strength may be a key element to the success or failure of protective equipment in relation to these injuries. Twenty six healthy male adults (14 Caucasian (C), 12 African Caribbean (AC); mean age (21±1.7yrs) were matched for height (1.79±.08cm) and mass (84±16.4kg). All participants had bone mineral density (BMD) measurements obtained by a Discovery QDR DXA scanner (Hologic Inc, USA). Measurements were taken at the lumbar spine (LS), femoral neck (FN) and facial sites; mandibular ramus (R) and body (Mb). Multivariate statistical analysis was conducted on both groups. Mean BMD for R were 0.65 ±0.28 g/cm^2 for C and 0.92±0.25 g/cm^2 for AC, Mb were 1.40±0.34 g/cm^2 for C and 1.45±0.36 g/cm^2 for AC. A significant ($F=3.752$, $p<.05$) ethnicity affect was detected across all BMD sites with the highest significance at the ramus ($p<.0125$). Correlations between BMD for the two facial sites were not statistically significant for the C group ($r=.323$, $p>.05$), but was for the AC group ($r=.636$, $p<.05$). Whole body analyses showed no significant correlations ($p>.05$) between facial sites and FN or LS BMD for either cohort. Mean R and Mb was higher in the AC than C group but both demonstrated high and low values. Mandibular BMD has previously been assessed in osteoporosis and implant design studies. Thus, individuals with lower BMD could be more susceptible to facial injury, particularly if they do not wear facial protection or compete in sports where weight maintenance is important. Our findings highlight that some individuals may be more susceptible to facial injury and therefore, facial protection in sport should be worn and more customised to the individual.

D

Reference	Country	Device details	Region of dentition	Bite Force (N)±(S.D)	Gender [n]	Age (years)±(S.D)	Ethnicity
Childhood Bite Force Papers (<18years)							
(Pereira et al., 2007)	Brazil	Pressurised rubber tube transducer.	Bilateral 1st molar: Mixed or permanent dentition	356.98±48.13	Boys mixed [32]*	9.5 ± 1.48 yrs	Not reported
				345.73 ± 41.44	Girls mixed [21] †	8.81 ± 1.25 yrs	
				387.36 ± 27.60	Boys permanent [23] *†	13.13 ± 2.24 yrs	
				361.10 ± 38.40	Girls permanent [25]	13.44 ± 2.32 yrs	
(RoldÁn et al., 2009)	U.S.A	Dual beam unidirectional transducer	Mean of right & left unilateral molar (Mo) & Incisors (In)	Mo 50 In 200	Mixed m & f [7]	5 yrs	Not reported
				Mo 150 In 300	Mixed m & f [7]	8 yrs	
				Mo 250 In 450	Mixed m & f [7]	11 yrs	
				Mo 350 In 600	Mixed m & f [7]	14 yrs	
(Varga et al., 2011)	Croatia	Hydraulic pressure gauge	Mean of right & left unilateral molar	522 ± 181	Male <18 [15] *	15 yrs	Caucasian
				465 ± 234	Female <18 [15]	15 yrs	
				777 ± 78	Male ≥18 [14] *†	18 yrs	
				481 ± 190	Female ≥18 [16]†	18 yrs	
Multiple Age Range Bite Force Papers							
(Bakke et al., 1990)	Denmark	Recorder, Chart recorder and amplifier	Unilateral 1st molars	356.9 ±64.3	M [8] F [9]	5-10	Not reported
				475.5 ±86.9	M [10] F [10]	11-20	
				572.3 ±72.2	M [9] F [11]	21-30	
				M 530.5 ±97.5 F 432.6 ±74.9	M [10] F [10] *m/f	31-40	
				M 610.8 ±131.1 F 469.9 ±126.3	M [9] F [11] *m/f	41-50	
				M 538.3 ±96.9 F 409.4 ±128.8	M [10] F [7] *m/f	51-60	
				373.8 ±138.2	M [3] F [5]	61-70	
(Palinkas et al., 2010)	Brazil	Digital dynamometer	Unilateral 1st molar (mean of right & left presented)	M185±30 F163±30	Mixed M [20] F [20]	7-12 yrs	Brazilian
				M405±30 F280±31	Mixed M [20] F [20] *	13-20 yrs	
				M344±31 F234±31	Mixed M [20] F [20] *	21-40 yrs	
				M323±34 F202±31	Mixed M [20] F [20] *	41-60 yrs	
				M372±49 F162±46	Mixed M [8] F [9] *	61-80 yrs	

Reference	Country	Device details	Region of dentition	Bite Force (N)±(S.D)	Gender [n]	Age (years)±(S.D)	Ethnicity
Adult Bite Force Papers (>18years)							
(Bakke et al., 1989)	Denmark	Strain gauge transducer	Unilateral (Uni) and bilateral (Bi) 1st molars	Uni. 480 ±163 Bi. 347 ±132	M [8] F [11]	29 ± 11yrs (20-60 range)	
(Braun et al., 1995a)	Austria	Pressurised tube transducer	Bilateral Molars	814 ± 209 615 ± 138	Male [86] * Female [56] *	32.4 yrs (26-41 range)	Not reported
(Burnett et al., 2000)	Northern Ireland, UK	Miniature load cell and strain gauge transducer	*Migrainers* Bilateral 1st molar	455.9 ±82.9 232.5 ±68.8	Mig. M [1] F [9] *Mig Cont. M [1] F [9] *	43 yrs (29-51 range)	Not reported
(Caloss et al., 2011)	U.S.A	*Dentures* Dual beam transducer	Unilateral (right side only)	Pre-molar 105± 38.7 Molar 112± 43.5	Male [7] Female [10] Pooled	60.3 ± 13.0 yrs	Not reported
(Ferrario et al., 2004)	Italy	Stainless steel strain gauge transducer	Molar to incisors, each tooth separately	M 146±44 F 94±38 M 139±51 F 96±37 M 190±79 F120±43 M 254±72 F179±77 M 291±57 F206±87 M 306±42 F235±71 M 294±56 F222±73	Central Incisor Lateral incisor Canine 1st premolar 2nd premolar 1st molar 2nd molar	Male [36] * 20.3 ± 2.2 yrs Female [16] * 20.1 ± 1.1 yrs	White Northern Italian
(Kiliaridis et al., 1995a)	Sweden & Saudi Arabia	Metal fork like device using strain gauges	2nd premolar/1st molar. Worn dentition.	651 ± 196 556 ± 218	Male [30] * submax Female [24] *	Mean 40 yrs Mean 28 yrs	Not reported
(Kiliaridis et al., 1995c)	Sweden & Greece	Metal fork like device using strain gauges	2nd premolar/1st molar. Experimental & control groups	Exp. 565 ± 171 Cont. 702 ± 183	Male [7] Female [10] Male [4] Female [4]	20-31 yrs	Not reported

Reference	Country	Device details	Region of dentition	Bite Force (N)±(S.D)	Gender [n]	Age (years)±(S.D)	Ethnicity
(Lepley et al., 2011)	U.S.A	Custom made transducer	Unilateral right 2nd premolar (P) & 1st molar (M).	P 373.8 ±102.6 M 383.9 ±102.3	Male [15]	22-32 yrs	Not reported
				P 314.7 ±96.5 M 338.7 ±113.8	Female [15]		
(Motegi et al., 2009)	Japan	Pressure sensitive film (Force = contact area X pressure)	Whole occlusal surface.	942.9 ±440.1	M [22] F [24]	>60s	Not stated – presumed Japanese.
				890.3 ±273.3	M [28] F [24]	>80s	
(Müller et al., 2001)	Germany	Load cell with full bridge strain gauge	*Dentures* Bilateral molar	Old teeth 142.9	Male [6] Female [3]	74.2 ±5.5 yrs	Not reported
				New teeth 102.2			
(Paphangkorakit and Osborn, 1997)	Canada	U shaped multidirectional transducer	Incisal (comparing jaw opening height)	@ 9mm jaw opening 233 ±65	M [8] F [2]	28-36 yrs	Not reported
(Regalo et al., 2008)	Brazil	Digital dynamometer	Molar (Mo) mean R&L and incisor (Is)	M Mo 502 ±47 M Is 206 ±24 F Mo 272 ±34 F Is 140 ±20	M [28] F [13] *Molar & incisors	18-28 yrs	Indigenous* incisors
				M Mo 484 ±53 M Is 150 ±18 F Mo 288 ±50 F Is 93 ±15	M [28] F [13] * Molar & incisors	18-28 yrs	White Brazil* incisors
(Shinogaya et al., 2001)	Japan & Denmark	Pressure sensitive film	Whole occlusal surface.	1650.8 1616.9 1100.7	Senior Male [10] *MPa Young Male [12] *†N Young Female [12]† ■	55.5 yrs 22 yrs 21 yrs	Japanese
				1042.0	Young Female [12]■Mp	22.5 yrs	Danish
(Sondang et al., 2003)	Japan	Dental prescale system (Fuji film)	Whole occlusal surface.	806.2 ±324.8	Female [64]	21.9 yrs	Not stated – presumed Indonesian

Reference	Country	Device details	Region of dentition	Bite Force (N)±(S.D)	Gender [n]	Age (years)±(S.D)	Ethnicity
(Thompson et al., 2001)	U.S.A	Unidirectional transducer	Unilateral premolars. Experimental & control groups	Exp. 546 ±137	M [7] F [7]	22-35 yrs	Not reported
				Cont. 520 ±190	M [7] F [7]		
(Tortopidis et al., 1998a)	Scotland	Stainless steel beam and strain gauge transducer	Anterior, canine to canine	339 ±60.8 (274-440 range)	Male [9]	30 yrs (25-35 range)	Not reported
(Tortopidis et al., 1998b)	Scotland	Stainless steel beam and strain gauge transducer	Unilateral p/m	428.5 ±132	Male [8]	29 yrs (25-32 range)	Not reported
			Bilateral p/m	579.3 ±235			
			Ant. Incisors	286.7 ±164			
(Tortopidis et al., 1999)	Scotland	T- shaped, stainless steel beam and strain gauge transducer	Bilateral *Edentulous* premolars	Healthy 115 ±42	No gender definition Healthy [11] TMD [10]	67 yrs (64-75 range)	Not reported
				TMD 75 ±22			
(Van Der Bilt et al., 2008)	Netherlands	Strain gauge transducer	Unilateral and bilateral molars	Uni. 490 ±192 Bi. 652 ±151	Male [13]*Bilateral	37 ±16	Not reported
				Uni. 418 ±138 Bi. 553 ±170	Female [68]*	39 ±14	

E

Factors Affecting Craniofacial Injury – PhD experimental study

Participant Questionnaire

Please complete this questionnaire honestly and to the best of your knowledge, your details will be treated as confidential and nobody other than the primary investigator will be able to trace these details back to you.

Personal Details

Date of birth: *(dd/mm/yy)* ___ / ___ / ___ Height (m): _____ Weight (Kg): _____

Sex: *(please tick)* Male ☐ Female ☐

How would you best describe your ethnicity? *(please tick)*

☐ White British ☐ Asian or Asian British – Chinese ☐ Black or Black British – Caribbean

☐ White European ☐ Asian or Asian British – Bangladeshi ☐ Black or Black British – African

☐ Asian or Asian British – Pakistani

☐ Asian or Asian British – Indian ☐ Other Ethnic Background

- *If other, please indicate* _____

Do you smoke? *(please tick)* Yes ☐ No ☐ Previously ☐

- *If yes or previously, how many a day?* _____ *For how many years?* _____ *How long ago?* _____

Do you drink alcohol? *(please tick)* Yes ☐ No ☐ Previously ☐

- *If yes or previously, how many units a week?* (a standard 175ml glass of white, red or rosé wine = 2.3 units, a pint of cider= 2.6 units, a pint of larger = 2.3 units and a single 25ml measure of spirit = 1 unit)

_____

How often do you take part in physical activity (*walking, gardening or vigorous housework*) or exercise per week?

(please tick) (*Please describe the type of exercise you take part in)*

☐ Less than 1 hour

_____

☐ 1+ hour ☐ 2+ hours

_____

☐ 3+ hours ☐ 4+ hours

_____

☐ 5+ hours

Have your diet or physical activity levels ever changed dramatically? *(please give details)*

_____

Medical History

Do you suffer from migraines? *(please tick)* Yes ☐ No ☐ Previously ☐

Have you or a close family member been diagnosed with a bone disorder or disease such as osteoporosis or temporomandibular disorder (TMD)? *(please tick)* Yes ☐ No ☐

- *If yes, please give details*

_____

Have you ever broken a bone in your body? *(please tick)* Yes ☐ No ☐

- *If yes, please give details as to how, which bone and when*
  _____

  _____

Have you ever had a scan or X-ray of your bones/ a bone? *(please tick)* Yes ☐No ☐

- *If yes, please give details as to why, which bone and when*
  _____

Have you been prescribed medication for a period of time greater than 12 weeks? *(please tick)* Yes ☐ No ☐

- *In particular, have you ever needed medication for heart disease or diabetes? Or have you taken steroids, anti-histamines or anti-inflammatory tablets for a period longer than 12 weeks? (please give details)*

_____

_*____*

Female Participants only

Do you use a form of hormonal contraception (i.e. the pill/ implant)? *(please tick)* Yes ☐ No ☐ Previously ☐

-If yes or previously, please indicate which and for how long you used it?

_____

Do you have regular periods? (i.e. a period every month for the past six months) *(please tick)*

Yes ☐ No ☐ Previously ☐

Have you had a period in the last 12 months? *(please tick)* Yes ☐ No ☐

Are you taking hormone replacement therapy (HRT) medication?

- *If yes, please give details as to how long* _____

_*____*

Dental History

Have you ever received orthodontic treatment? *(please tick)* Yes ☐ No ☐

- *If yes, how long ago did you receive treatment and for what purpose?*

  _____

Have you ever had dental surgery? *(please tick)* Yes ☐ No ☐

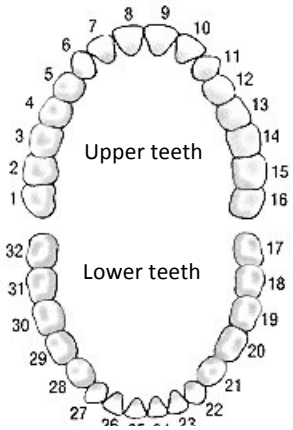
- *If yes, how long ago did you receive treatment and for what purpose?*

  _____

Do you have any false dentition, missing teeth or dental reconstruction?

(i.e. veneers, crowns, bridgework?)

(please tick) Yes ☐ No ☐



- *If yes, please give details and indicate on the chart to the right* _____

  _____

When did you last visit your Dentist? *(please tick)*

3 months ☐6 months ☐12 months ☐ Longer than 12 months ☐

- *Did you require treatment at your last visit? Did the dentist identify a weakness in your teeth? Please give details*

  _____

_____

Do you experience any pain in your mouth when chewing, talking or swallowing? *(please tick)*Yes ☐ No ☐

Do you suffer from Bruxism or a similar disorder that includes teeth grinding, tapping or clenching during sleep? *(please tick)* Yes ☐ No ☐

- *If yes, how long have you experienced this?*

  _____

Have you ever sustained an injury to your mouth, face or head? *(please tick)* Yes ☐ No ☐

- *If yes, please give details as to where and how long ago*

  _____

Have you ever had a scan or an X-ray of your facial bones/teeth? *(please tick)* Yes ☐ No ☐

- *If yes, please give details*

  _____

Have you ever had a facial osteotomy? *(please tick)* Yes ☐ No ☐

- *If yes, please give details as to how long ago*

  _____

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